Volume 9B, Number 2–3, July 1960

BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

Section B ZOOLOGY

Bull. Res. Counc. of Israel. B. Zoology

Volume 9B, Number 2–3, July 1960

BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

Section B ZOOLOGY

Bull. Res. Counc. of Israel. B. Zoology

BULLETIN
OF THE RESEARCH COUNCIL
OF ISRAEL

—

MIRIAM BALABAN
Editor

—

NOTICE TO CONTRIBUTORS

Contributors to the *Bulletin of the Research Council o' Israel* should conform to the following recommendations of the editors of this journal in preparing manuscripts for the press.

Contributions must be original and should not have been published previously. When a paper has been accepted for publication the author(s) may not publish it elsewhere unless permission is received from the Editor of this journal.

Papers may be submitted in English and in French.

MANUSCRIPT

General

Papers should be written as concisely as possible. MSS should be typewritten on one side only and double-spaced, with side margins not less than 2.5 cm wide. Pages, including those containing illustrations, references or tables, should be numbered.

The Editor reserves the right to return a MS to the author for retyping or any alterations. Authors should retain copies of their MS.

Spelling

Spelling should be based on the Oxford Dictionary and should be consistent throughout the paper. Geographic and proper names in particular should be checked for approved forms of spelling or transliteration.

Indications

Greek letters should be indicated in a legend preceding the MS, as well as by a pencil note in the margin on first appearance in the text.

When there is any room for confusion of symbols, they should be carefully differentiated, e.g. the letter "1" and the figure "1"; "O" and "0".

Abbreviations

Titles of journals should be abbreviated according to the *World List of Scientific Periodicals.*

Abstract

Every paper must be accompanied by a brief but comprehensive abstract. Although the length of the abstract is left to the discretion of the author, 3% of the total length of the paper is suggested.

References

In Sections A and C, and in Letters to the Editor in all Sections, references are to be cited in the text by number, e.g. . . . Taylor[3] . . . , and are to be arranged in the order of appearance.

In Sections B, D, E, and G, the references are to be cited in the text by the author's name and date of publication in parentheses, e.g. .... (Taylor 1932).... If the author's name is already mentioned in the text, then the year only appears in the parenthesis, e.g. ... found by Taylor (1932).... The references in these Sections are to be arranged in alphabetical order.

In Section F, references are to be cited in the text by number in square brackets, e.g. . . . Taylor [3]. . . , and are to be arranged in alphabetical order.

The following form should be used:

3. TAYLOR, G. I., 1932, *Proc. roy. Soc.*, A138, 41.

Book references should be prepared according to the following form:

4. JACKSON, F., 1930, *Thermodynamics*, 4th ed., Wiley, New York.

TYPOCRAPHY

In all matters of typography, the form adopted in this issue should be followed Particular attention should be given to position (of symbols, headings, etc.) and type specification.

ILLUSTRATIONS

Illustrations should be sent in a state suitable for direct photographic reproduction. Line drawings should be drawn in large scale with India ink on white drawing paper, bristol board, tracing paper, blue linen, or blue-lined graph paper. If the lettering cannot be drawn neatly by the author he should indicate it in pencil for the guidance of the draftsman. Possible photographic reduction should be carefully considered when lettering and in other details.

Half-tone photographs should be on glossy contrast paper.

Illustrations should be mounted on separate sheets of paper on which the caption and figure number is typed. Each drawing and photograph should be identified on the back with the author's name and figure number.

The place in which the figure is to appear should be indicated in the margin of the MS.

PROOFS

Authors making revisions in proofs wil be required to bear the costs thereof. Proofs should be returned to the Editor within 24 hours; otherwise no responsibility is assumed for the corrections of the author.

REPRINTS

Reprints may be ordered at the time the proof is returned. A table designating the cost of reprints may be obtained on request.

Orders in America should be addressed to The Weizmann Science Press, P.O.B. 801, Jerusalem, or through booksellers and in England and Europe to Wm. Dawson and Sons, Ltd., Cannon House, Macklin Street, London. W. C. 2, direct or through booksellers —Annual subscription per section (four issues): IL. 6.000 ($6.00, £2.20). Single copy: IL. 1.50 ($1.50, 12s.) — Manuscripts should be addressed: The Editor. The Weizmann Science Press of Israel, P.O.B. 80 Jerusalem, 33 King George Ave. Telephone 62844

STUDY ON *Paramphistomum microbothrium* FISCHOEDER, 1901

A RUMEN PARASITE OF CATTLE IN ISRAEL

J. LENGY*

Department of Parasitology, The Hebrew University of Jerusalem

ABSTRACT

The complete life-cycle of *P. microbothrium* Fischoeder, 1901, a rumen parasite of cattle in Israel, is described; anatomical details of all intermediate stages are presented in their gradual development. New light is shed on the nature and function of the so-called "primitive gut" in the miracidium; this structure is shown to be a true penetration gland; the term "apical gland" is adopted for it. Factors influencing the development of larval stages of the trematode are discussed. The penetration of the miracidium into the intermediate host snail is described in detail.

Alternate production of daughter-rediae and cercariae is described and discussed. Cercarial features hitherto unrecorded for paramphistome cercariae, such as a "mucoid fin" on the tail and sensory bristles on the body and tail, are described. The excretory system of the cercaria is analyzed; the terms "concrement ducts" and "excretory concrements" are proposed for the descending, lateral excretory ducts and the globular bodies, within them, respectively. A technique for the collection of large numbers of metacercariae is offered. The validity of *P. microbothrioides* as a species apart from *P. microbothrium* Fischoeder 1901 is questioned. A key to the species of genus *Paramphistomum* is presented.

INTRODUCTION

ere are many references in the literature to epidemics in cattle in different parts of world, caused by trematodes belonging to the family Paramphistomidae. This nily comprises many species and each geographical region has one or several ecies which are apparently adapted to local conditions.

In Israel, sporadic outbreaks of paramphistomiasis among cattle are not unmmon (Nobel, 1956). One such outbreak in the Hadera region was brought to our ention about three years ago. On the basis of material obtained from several ested cows we were able to identify the pathogen as *Paramphistomum microthrium.*

The purpose of the present study was to trace the life-cycle of *P. microbothrium* the ecological conditions of Israel, and, in particular, to determine its development the local snail which serves as its intermediate host. The life-cycles of several ecies of genus *Paramphistomum* have been worked out in detail in different parts the world. The type of cercaria characteristic for his genus is known. Preliminary

* This paper is part of a doctorate thesis carried out in the Parasitology Deptarment of the Hebrew iversity of Jerusalem. It is supported by a grant from the Union of Jewish Women of South ica. The helpful interest shown by Prof. G. Witenberg in this study is gratefully acknowledged.

ceived January 18, 1960

investigations had shown that *Bulinus truncatus*, a species of fresh-water snail pre lent in Israel, frequently harboured paramphistome cercariae. We subsequen succeeded in proving that these cercariae were the larval forms of *P. microbothriu*

MATERIALS AND METHODS

Parasite ova obtained from a cow in the Hadera region, Israel, served as the ini material for experiments. The presence of paramphistome ova in the cow's fae was established by a Telemann concentration test. In order to obtain the egg co centrate, the diluted faecal material was filtered through a no. 25 bronze wire me washed in several changes of tap water by sedimentation, and the final, almost muc free filtrate containing the ova was allowed to settle on the bottom of a gallon j This jar was subsequently kept at a temperature of 28°C and the supernatant wa was periodically changed to keep it transparent. Under these conditions, miraci emerged on the 17th day of incubation.

Laboratory-reared *Bulinus truncatus* snails were infected experimentally by plac them individually in 3x1 inch glass vials containing between 5 and 20 newly emerg miracidia. At the end of eight hours the snails were transferred to aquaria wh they were kept at a temperature of 28°C.

Cercaria of *P. microbothrium* began to emerge from infected snails as early a days after exposure of the snails to miracidia. The cercariae were allowed to enc on lettuce leaves or various water vegetation which were subsequently kept in wa for about a week to allow for optimal development of the metacercariae before be fed to two paramphistome-free sheep. Each sheep received a predetermined num of metacercariae.

The first sheep was slaughtered 48 days after infection and immature forms *P. microbothrium* were recovered from its rumen and reticulum. The second she started passing paramphistome ova 89 days after the infected feeding. It was slaug tered 16 months after infection and sexually mature forms of the trematode w recovered from its rumen and reticulum. Both immature and mature worms w fixed in Bouin, embedded in paraffin, and cut in serial sections. In addition, medi sagittal sections of adult worms obtained from the rumen of a naturally-infect cow were used for purposes of comparison. Final identification was based on h tological details of acetabulum, "pharynx" and genital atrium, in accordance w Näsmark (1937).

The behaviour and movements of the various larval stages were observed und a dissecting microscope while anatomical details were studied with the aid of ordinary microscope and a phase microscope. Larval stages developing within t intermediate snail host were dissected out of the snail and studied alive either u stained or stained with vital stains. Whole mounts of trematode specimens at vario stages of development fixed in Bouin and stained with alum carmine were also use The stains found best for the study of larval stages were neutral red, eosin, brillia cresyl blue, Nile blue sulfate, methylene blue and gentian violet. Observation

ing cercariae in 0.2% solution of gentian violet, or diluted India ink were helpful the study of non-staining external tail structures. A drop of 1% eosin solution ded to a drop of diluted India ink was found useful in bringing out details of the cretory and digestive systems of the cercariae after an exposure of about a quarter an hour. In the study of chemotactile hairs, the live cercariae were placed under a ver slip in a drop of tap water mixed with a drop of 5% aqueous methyl cellulose d observed at room temperature or in mammalian serum warmed to 30°C, der a 97 × oil-immersion objective of an ordinary microscope. Supplementary servations of chemotactile hairs were made with a phase microscope, using the gh-dry objective.

Size measurements of the various larval stages were taken wherever practicable unstained, unmounted specimens. Miracidia and cercariae, because of their gorous activity, required slowing down under pressure of cover slip in a 2–3% lution of methyl cellulose. To obtain measurements of internal structures, it was ten necessary to use cover slip pressure and thus somewhat flatten the specimens. me structures could best be seen only in whole mounts where a certain amount shrinkage had occurred. All size measurements were based on a minimum of ten servations.

EGG

The egg is oval, operculated, smooth-surfaced and milky to brownish in colour. It easures 167(149–178)μ in length and 98(91–109)μ at its widest diameter. The oper-lum measures an average 25μ in diameter, the opercular line situated some 3μ from e anterior end. A slight projection is situated asymmetrically at the posterior le of the egg. In fresh bovine or ovine faeces, the egg is usually found in the morula age with 40–50 vitelline cells surrounding the embryo. When freshly laid in vitro, may contain an uncleaved ovum or be in an early segmentation stage (4–8 meta-eres).

DEVELOPING EMBRYO

Eggs prepared for hatching were observed daily under high power magnification til emergence of the miracidia. The transparency of the shell permitted observations intact eggs. After the ensuing segmentation within the first 4–5 days, the mbryo is oval, 51–65μ in diameter, and surrounded by numerous yolk cells. It is cated somewhat posterior to the center of the egg. From the sixth to eighth day, marked changes occur except for an elongation of the embryo with a shift to a ghtly anterior position and a decrease in the number of yolk cells. On the ninth y of development the embryo measures an average 79 x 55μ, and consists of a ass of large, hexagonal, nucleated cells with epidermal plates forming around it.

The 10th and 11th day of development is marked by rapid changes in the embryo. now measures 117 x 58μ, and shows the epidermal plates arranged in four trans-rse rows, ciliated and clearly visible. These plates range between 25–35μ in length,

those of the second row being somewhat longer than the rest. An apical papilla an rudiments of the apical gland* ("primitive gut") are first seen on the tenth day the former surrounded by a mucoid plug (Barlow, 1925) in the anterior end of th egg. Two pairs of "penetration glands", one on each side of the apical gland, ca also be traced. A pair of flame cells, located laterally near the juncture of the secon and third rows of epidermal plates, becomes visible on the 11th day. Germinal cell form in the posterior half of the embryo. Occasional transverse contractions of th developing embryo are noticeable.

By the 13th day, the miracidium measures an average 141 x 58μ and is quite active contracting its body both at gut level and juncture of third and fourth rows of epider mal plates, with corresponding contractions and extensions of the apical gland Both flame cells are prominent and beating vigorously. An excretory duct may b seen leading posteriorly from each flame cell till about two thirds the length of th body from the anterior end, then looping anteriorly up to the posterior margin o the apical gland where it coils once more posteriorly. The ducts empty laterall opposite each other at the juncture of third and fourth epidermal plates. A singl embryo ball is formed in the posterior third of the body. The vitelline cell which had already undergone a great reduction in number are now practically al absent, the developing miracidium appearing to be surrounded by a semi-liqui medium arranged in a few large compartments immediately under the egg shell, th most anterior of which comprises the mucoid plug. The latter is reduced and just fil the operculum.

On the 14th day of development, the opercular line can barely be seen. The egg i yellow-green in colour and semi-opaque at the periphery. The developed miracidiu measures 155 x 55μ, just about filling the length of the egg. It is quite active, with th cilia on the epidermal plates beating. The mucoid plug is greatly reduced or entirel absent, the apical papilla in the latter case appearing to be pressed right against th inner wall of the operculum. A brain mass some 20 μ in diameter is discernible jus posterior to the apical gland which now measures around 45μ in length when relaxed

On the 15th day, the miracidium measures 175 x 55μ and fills the entire length o of the egg, the tail at times folded slightly for lack of space. The miracidium is very active and seems about ready to hatch. Its activity follows a more or less definit pattern. General bodily contractions lasting around eight minutes are followed by a series of violent jerks of the anterior third of the body, the apical papilla being used to push against the center of the operculum. After 10–15 seconds of this, the cilia at one side of the apical papilla are placed against the operculum, and all move ment ceases for about a minute. This process is repeated with increasing rest period in between. The pattern of activity just described persists intermittently till the hatching of the miracidium on the morning of the 17th day.

By the 16th day the miracidium measures 193 x 62μ in the relaxed state

* For details, see description on p. 10.

The mucoid plug is completely dissolved. Small granular conglomerations appear within the colloidal substance surrounding the miracidium. The apical gland, when active, extends to about half the length of the miracidium. Under the oil immersion objective of a phase microscope, six minute ducts can be seen traversing the apical papilla and opening to the outside. Four of these, lying laterally, two on each side of the apical papilla, can be traced as ducts of the penetration glands while the remaining two, situated medially to the others, are the anterior extensions of the apical gland. Granular material from the latter seems to be introduced into these minute ducts with each extension and contraction of the organ.

HATCHING OF THE MIRACIDIUM

To observe the hatching process, some eggs were placed in water in a Syracuse dish and observed under a dissecting microscope while others were placed under cover slip and studied with the high power objective of an ordinary microscope. The latter procedure, though allowing for more detailed observation, invariably hampered or prolonged the process of hatching.

Forty eight hours prior to the hatching, the miracidium shows a gradual but marked decrease in its activity. The miracidium may now be found resting for long intervals, its apical papilla pressed directly against the operculum, with just occasional extensions and contractions of its body.

Actual hatching is initiated with the opening of the opercular cap. Short seconds before this happens, there is a shiny translucence to the opercular region, the opercular line becomes sharp and distinct, and the operculum seems to swell slightly and push off from the rest of the shell. Next, it may either detach completely and fall away from the shell, or, as occurs more often, unhinge at one end only and push outwards and sideways in door-like fashion. It has not been ascertained whether or not the miracidium actively participates in the opening of the operculum. In some cases the apical papilla of the miracidium may be seen in contact with the operculum right through its opening, to all appearances actually pushing off the operculum. In other cases, however, the miracidium withdraws its apical papilla just prior to the opening of the operculum and no part of its body is in contact with the operculum at the moment of its opening.

The rim of the operculum appears serrate or finely-toothed. Similar dentations may be found on the egg end of the opercular opening, wich measures a bare 30-35μ in diameter. Once the operculum is pushed off, the miracidium quickly introduces its apical papilla through the opercular opening and proceeds to emerge by rapid extensions and contractions of the body coupled with vigorous beating of the cilia. The cilia on the part of the body still within the egg are seen beating more actively than those covering the free anterior part. If for some reason the miracidium dies in the midst of hatching, the extruded portion of its body is forced back into the shell. It should be noted that the width of the relaxed miracidium is just about twice the

diameter of the opercular opening. The actual escape of the miracidium is effecte in 50–90 seconds.

Rowan (1956), in studies of *Fasciola hepatica*, observed the expulsion of a viscou material concurrent with the hatching of the miracidium. In the present study, n viscous material precedes or envelops the miracidium upon escape, and once full free, the miracidium immediately swims off.

FACTORS INFLUENCING THE DEVELOPMENT OF THE MIRACIDIUM

Investigation of the life-histories of many trematodes by numerous authors ha shown that the rate of development of the miracidium and its hatching depend o various environmental factors. In the present study observations were made concernin the effect of temperature, light and seasonal changes on the developing miracidium Summarized briefly, these were:

1. The optimum temperature for development was in the vicinity of 28°C. At tha temperature miracidia emerged after an incubation period of 16–18 days. A room temperature (20–24°C) the incubation period was extended to 21 days. A a temperature range of 15–18°C, the incubation period was as long as 29 days an an appreciable percentage of the eggs failed to hatch.

2. Light does not seem to have any effect on the development of the miracidium Eggs incubated in the dark hatched at the same time as eggs exposed to consta light, temperatures being equal. However, the hatching process itself seemed t be triggered off by light. When full-term eggs were placed under a light sourc in large glass vials half filled with tap water, escaped miracidia could be found i the water after 10–20 minutes of exposure to light. When the procedure wa repeated in total darkness, no miracidia appeared in the water even after severa hours.

3. Even under constant artificial light, miracidia emerged mainly during the earl morning hours. No explanation could be offered for this remarkable phenomenon.

4. Seasonal variations in the rate of development were noted, the miracidium takin up to twice as long to develop during the winter months. However, eggs kept under constant conditions of temperature hatched at comparable rates all the year round.

5. Within any egg culture, not all eggs hatched on the same day. While some eggs hatched on the 17th day at 28°C, others hatched as late as the 27th.

MIRACIDIUM

Freely swimming miracidia in a Syracuse dish filled with tap water are pyriform or bullet-shaped ciliated organisms. They may swim in straight lines along the bottom of the dish or may zig-zag, rising very gradually to the water surface then gradually

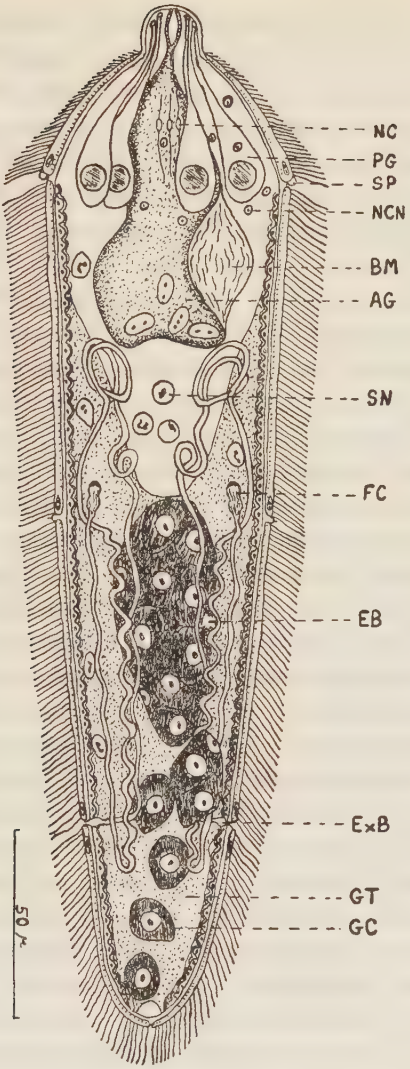


Figure 1
Miracidium, optical section*

escending again. Some are seen swimming clockwise or counterclockwise along he rim of the dish. Still others may be found swimming around in short, agitated ircles. Fresh miracidia are rapid swimmers (55 mm, the dish diameter, in 22–25 econds) and almost constantly on the move. Any change in the direction of miracidial notion, as necessitated by obstacles in its way, is achieved smoothly and without ny pause. Miracidia may, on rare occasions, pause at the edge of the dish in an angle erpendicular to the periphery and remain there, cilia beating vigorously while the

* For the meaning of the abbreviations, see p. 59.

posterior half of the body lashes from side to side. While swimming, the miracidia also exhibit a slow piral motion. This motion was described by Bennett (1936) as counterclockwise. In the present study, both clockwise and counterclockwise spiralling miracidia were observed, though the latter predominated.

Newly emerged miracidia in a glass vial filled with water and placed under a strong light tend to congregate near the surface of the water which is the point closest to the light source and also the warmest. Thus, they exhibit positive phototropism or possibly thermotropism.

The miracidium, as mentioned previously, is a pyriform, ciliated organism, measuring an average 270(209–301) μ in length. Its widest diameter occurs roughly at $^1/_6$ the body length from the anterior end and measures 54 (44–66) μ across. Posterior to this point, the body of the miracidium tapers gradually, measuring 47(33–66) μ in diameter at the middle, and 26(22–29) μ at the evenly-rounded tail end. These measurements apply to freshly hatched miracidia only. Eight hours after hatching, the miracidium may shorten and broaden considerably and measure around 150 x 100 μ, becoming gourd-shaped, triangular, or, before death, even spherical.

At its anterior extremity, the miracidium bears a blunt, smooth apical papilla (also called "terebratorium") which, in the relaxed miracidium is somewhat wider than long, approximately 11 × 15 μ, and devoid of cilia. The miracidium is capable of protracting or retracting the apical papilla. Under pressure of the cover slip, miracidia are quite often observed with the apical papilla in the retracted state.

The body of the miracidium, excluding the apical papilla, is covered with twenty flat, ciliated epidermal cells or plates which are arranged in four transverse rows. These epidermal cells have been fully described by Bennett (1936) and others (Durie 1953; Tandon 1957) for various paramphistomid miracidia, and need not be dealt with here in detail. In number, arrangement and position of their nuclei, the epidermal cells on the miracidium are in close agreement with those described by Bennett (1936) for the miracidium of *P. microbothrioides* (determined by that author as *Cotylophoron cotylophorum*). There are some size differences, however. The first, most anterior row encircles roughly $^1/_8$ of the body and consists of six triangular cells, their apices converging at the base of the apical papilla while their bases form the shoulder-line of the miracidium. The second row consists of eight long, narrow, rectangular cells that encircle roughly $^1/_3$ of the body. The four cells of the third row, though rectangular, are somewhat shorter and considerably broader than those of the second row and cover a little less than $^1/_3$ of the body. The fourth and final row consists of two roughly triangular cells, their apices posteriorly directed. They cover the posterior fifth of the body. The nuclei of the epidermal cells in the first, third and fourth rows are roughly bean-shaped and transversly elongated. Those of the second row are cross-shaped. The nuclei of all the rows except the fourth are situated very near the posterior borders of the cells, while those of the fourth are located near the anterior borders of their cells and are somewhat larger than the others.

The nuclei of the epidermal cells were studied by Bennett (1936) in living specimens

stained with intra-vitam stains. Durie (1953) observed nuclei in specimens stained with methylene blue. Tandon (1957), however, failed to stain the nuclei with anything but haematoxylin. The author, like Tandon, was unable to stain the nuclei with any of the common intra-vitam stains. In optical sections of the miracidia, however, the nucleus of each marginal epidermal eell could be traced fairly accurately. Only by the use of the oil immersion objeetive of a phase microscope was the author able to observe nuclei in surface view. Similar difficulties in observing the spaces between epidermal cells were resolved by use of the phase microscope. The very narrow (1–2μ) spaces between cells of the sa e row, and the somewhat wider spaces between two adjacent cell rows, were found to be filled with subepithelium from the underlying subepithelial stratum, and were devoid of cilia.

The epidermal cells are about 2–3 μ in thickness (those of the first row are somewhat thicker, especially at their bases) and are completely covered with cilia. The cilia are evenly long on all but cells of the first row where the cilia are shorter at the anterior borders of the cells and lengthen gradually posteriorly. The cilia on the posterior end of the body appear to be longer than the rest, but actual measurement fails to confirm this impression. In miracidia vitally-stained with brilliant cresyl blue or methylene blue, each cilium can be clearly seen airsing from a tiny basal granule located in the inner wall of the epidermal cell. In optical sections these basal dots form fine rows along the margins of the miracidial body.

Directly beneath the ciliated epidermal plates is a thin, transparent layer of subepithelium. This layer completely encircles the internal structures of the miracidium and extends anteriorly on the apical papilla. As was noted previously, the subepithelium pushes slightly through at the junctures between epidermal plates. This is especially noticeable at the junctures between rows of epidermal plates. Just before the death of the miracidium under pressure of a cover slip, the subepithelium pushes completely through at these junctures to form large globules filled with particles in Brownian movement. Though cell outlines cannot be observed in this layer, subepithelial nuclei can be seen widely dispersed throughout the body. The nuclei pick up vital stains nicely and appear elongated, oval or tear-shaped along the lateral margins of the miracidium, where they measure $7 \times 4\mu$, but more nearly circular in the center of the miracidium where they measure 5μ in diameter. The chromatin matter within the nuclei is polymorphic and may appear as an oval, concentric mass, an elongated eccentric mass or a series of minute masses tied together by fine chromatin lines.

Bennett (1936) and Durie (1953) described circular and longitudinal muscles as situated between the epidermal cells and the subepithelium. No such muscle fibers could be observed in the present study. However, immediately beneath the subepithelium, a narrow layer of closely packed but regularly wrinkled fibers could be seen running the length of the body along the sides, which was interpreted as longitudinal musculature. The minute spaces left betweon the longitudinal muscle fibers and the subepithelium, due to the waviness of the former, appeared to form a regular

pattern of small black dots. These apparently represent the loci of the circular muscle units.

The internal structures of the miracidium consist of an apical gland, penetration glands, a nervous system, excretory system and germinal tissue.

In the living miracidium, the apical gland (often called "primitive gut") is a prominent, saccular and extremely contractile structure in the anterior third of the body. In the extended miracidium it may reach posteriorly to about half the body length, assuming a roughly flask-shaped appearance, while in the contracted miracidium it flattens out and broadens, becoming more or less triangular and filling most of the central region in the anterior third of the body. Such variability in shape makes size measurements of this structure difficult and of little value. Anteriorly the apical gland narrows gradually till about the level of the base of the apical papilla where it flares out in two minute but distinct ducts. These ducts curve evenly outwards then inwards to meet and possibly merge just before opening medially at the surface of the apical papilla. Because of their minute size and the constant movement of the miracidium, the ducts are extremely difficult to observe and require patient and careful study. They were best seen when using the phase microscope. These ducts were somewhat easier to observe in the full grown but unhatched miracidium, but their openings at the surface of the apical papilla were partially obscured by the intervening shell matter. Bennett and Durie could see no opening to the exterior of the apical gland. Tandon (1957) maintained, "it opened by a narrow aperture at the head-papilla."

The apical gland is filled with granular matter which seems to move freely back and forth within the cavity of the structure. Tiny granules may also be discerned moving within its ducts. Four nuclei are situated in the posterior part of the gland. These are best seen just prior to the death of the miracidium when, under pressure of cover slip, the granular contents of the gland are forced out, leaving the nuclei clear to view. They are oval or pear-shaped and contain two or three small masses of chromatin in linear arrangement at their centers.

The nature and function of the apical gland have puzzled investigators for many years. Early writers believed it to be a vestigial gut. Recently the consensus of opinion has been to attribute glandular functions to this structure. Some authors (e.g. Durie, 1953) offer the fact that the "gut" supposedly has no opening to the exterior as evidence for its glandular nature. In the present study, however, the glandular nature of the "primitive gut" was ascertained. As will be shown presently, at least part of the granular contents of the "gut" are expelled through its ducts while the miracidium is in the process of penetration into the snail host tissues. It is not unlikely that the "primitive gut" is a true penetration gland while the so-called "penetration glands" are accessory glands that function in the hatching of the miracidium.

Two pairs of "penetration glands" are found in close juxtaposition to the apical gland. Posteriorly, they may extend a bit past the juncture of the first and second epidermal plate rows. Anteriorly, each sac-like gland opens in a narrow duct at the

sides of the apical papilla. The ducts of the "penetration glands" are easily distinguished from those of the apical gland by their large diameter and absence of any granular matter within them. The glands are grayish-transparent even in stained specimens and are rather difficult to observe with anything but a phase microscope. Each contains a single round nucleus, 5–8μ in diameter, whose chromatin mass is evenly distributed and diffuse and does not stain deeply with any of the vital stains. The "penetration glands" have been described by various writers as lying two on each side of the apical gland. Under certain conditions, this might be correct. The author, however, was never able to observe the glands in such an ideal position. They are mostly found medially oriented, dorsal to and superimposed over the apical gland. Before the death of the miracidium under pressure of the cover slip, the openings of the penetration ducts form minute protruberances on the surface of the apical papilla.

The nervous system of the miracidium consists of a central fibrous mass (designated by some as a "brain"), several neurons and two pair of sensory papillae. In the extended miracidium, the "brain" is a spindle-shaped, grayish mass of dense fibers which lies parallel to and immediately along the lateral posterior half of the apical gland. In the contracted miracidium, the "brain" assumes a quadrangular appearance and is situated along the posterior border of the apical gland, dorsal to it and partially overlapping it. It measures roughly 40 × 20μ. Though in no way attached to the apical gland, the "brain" seems to change its shape and position in accordance with the contractions and extensions of the former, and may be found on either side of it at varying intervals. No nerve cells or nuclei could be discerned within the mass of the "brain". However, numerous nerve cells or rather their nuclei were seen scattered anterior to the "brain" mass. These stain a deep blue with brilliant cresyl blue, measure approximately 3 μ in diameter and have a characteristic "bull's-eye" appearance.

Krull and Price (1932) observed three pairs of nerve cells in the miracidium of the frog's paramphistome, *Diplodiscus temperatus*. These were anterior to the brain, centrally-located and with terminations both at the base of the apical papilla and the brain mass. Bennett (1936) did not observe any such structures in the miracidium of "*Cotylophoron cotylophorum*". The present author, however, has on several occasions, noted nerve cells as described by Krull and Price. Their exact number could not be ascertained as they were seen for seconds at a time and did not pick up vital stains well. Three were seen during one observation period. They were located centrally, dorsal to the apical gland, approximately mid-way between the apical papilla and "brain" and were fairly parallel to one another. The nerve cells were non-staining, oval in shape and had tiny fibrils at both ends leading off anteriorly and posteriorly. The terminations at the base of the apical papilla could not be made out. However, they could be traced posteriorly as far as the brain mass.

A pair of sensory papillae are located laterally at the juncture between the first and second rows of epidermal plates. They originate from the latero-posterior border

of the epidermal cells of the first row, are dome-shaped and have tiny fibrils leading proximally to the central brain mass. A similar, somewhat smaller pair of papillae, each bearing a minute seta is present at the lateral junctures between the second and third rows of epidermal plates. These papillae, however, were extremely difficult to detect and were seen for brief seconds at a time. No nerve fibrils in connection with these papillae could be traced, and their sensory function could not be ascertained.

The excretory system of the miracidium consists of a pair of flame cells and their ducts. The flame cells are large, 10–15μ long, roughly hour-glass shaped and located laterally, one on each side of the body at the middle. Each posteriorly-beating flame cell opens into an excretory duct that winds posteriorly about a quarter of the way into the region of the fourth row of epidermal plates, then loops backwards and inwards and continues in tight coils anteriorly, curving gradually outwards at the level of the flame cell till about the middle of the second row of epidermal plates. There it makes a couple of tight loops around itself, turns inward and proceeds posteriorly, looping once more anterior to the flame cell and then continues fairly straight till it empties at the excretory pore which is situated laterally at the junctures between the third and fourth rows of epidermal plates. There is a widening in the excretory duct just before its terminus which may be regarded as a minute excretory bladder.

Roughly the posterior two thirds of the body constitute a cavity for which the name "germinal cavity" is proposed. Lined with a subepithelial layer, the germinal cavity contains a granular mass in which the germinal cells and embryo balls are embedded, the whole constituting the germinal tissue. The excretory ducts are superficially connected with the cell layer forming the germinal cavity. The exact boundaries of the latter could not be determined and were variable in different specimens. The germinal tissue is a very elastic mass which seems to have at least two points of attachment with the subepithelium along its posterior margin by means of fine, thin, contractile fibers. These fibers may be seen during the extension of the miracidium when the germinal tissue is pulled anteriorly, leaving a vacuole-like space between it and the subepithelium at the tail end. Similar vacuole-like spaces may occasionally also be found along the sides of the germinal tissue. The latter, however, is not attached to the subepithelium along its sides, though in close contact with it. At its anterior margin the germinal tissue usually leaves a free space between it and the apical gland. However, when the posterior margin of the extended apical gland comes in momentary contact with the anterior border of germinal tissue, the two become continuous and can hardly be distinguished one from the other. No cells could be seen in the granular mass of the germinal tissue along its anterior margin. The posterior and especially the middle portions of this tissue, however, were abundantly interspersed with germinal cells and one or two embryo balls. The former seemed to originate along the lateral margins of the layer, later to be detached and pushed centrally by newly developing cells, finally moving anteriorly to develop into embryo balls.

The germinal cells are irregular in shape, measuring 15–18 x 8–10μ. In miracidia stained intra-vitam, the nucleus of the germinal cells appears white against the background of the darker staining cytoplasm. The nucleus is circular about 5μ in diameter, and the chromatin is concentrated in the form of a dark mass in its center, giving the complete cell a distinct "bull's-eye" appearance.

Most miracidia were observed to contain one large embryo ball and another, somewhat smaller. Some contained only one; others, one large and two small. Where two or more embryo balls were present, the larger one was also the most anterior one. Large embryo balls consist of 16–20 closely packed cells surrounded by a thin membrane and may measure 65 $\times$ 25μ. Both germinal cells and embryo balls appear to move freely within the plastic germinal matrix during contractions or extensions of the miracidial body. Furthermore, the germinal tissue itself seems to move independently within the germinal cavity.

Longetivity of the miracidium

An experiment was carried out to determine the longevity of the miracidium in different liquid media and at varying temperatures. Equal numbers of freshly hatched miracidia were placed in Syracuse dishes containing: (a) distilled water; (b) tap water; (c) 0.6–1 % saline. The dishes were maintained at 28°C, 24° and 14°C. The experiment was started in the early morning hours. The dishes containing the miracidia were carefully inspected once every hour under a dissecting microscope and the number of dead miracidia in each dish was noted. Cessation of all movement, especially ciliary, on the part of the miracidium was designated as the death point. Results of the experiment showed:

1. Of the various media, tap water seemed to be the most favourable for the survival of the miracidium, with distilled water a close second and saline a fair third.

2. The optimal temperature for the survival of the miracidia was around 24°C. At this temperature some miracidia survived for as long as 17 hours in tap water, while at 14°C or 28°C, all miracidia were dead by the end of 12 hours.

Penetration of miracidium into snail

In order to observe penetration into the intermediate host, *Bulinus truncatus* snails were used as stated in the introduction. A large number of miracidia were placed in a finger bowl containing a few c.c. of tap water and a single young snail. Penetration attempts were studied through a dissecting microscope.

As was rightly pointed out by Durie (1953), the transparency of the shell and mantle tissues in the young snail facilitates close observation of the penetration process. Snails measuring about 2 mm in length were found best suited for this purpose.

Most miracidia become quite agitated when in the vicinity of the snail. Some,

however, exhibit no attraction to the snail. Many are equally attracted to snail faeca matter or the trail of slime left by the snail and concentrate around these material for long periods, making futile, clockwise boring motions, and completely ignorin the snail itself. When agitated, the miracidia proceed to swim around in short, elliptica courses rather than the customary straight lines. Occasionally, one may briefly touc the foot or tentacle of the snail and withdraw to resume its elliptical sweeps aroun the snail. Often, miracidia get under the snail's shell, probe around the dorsal wal of the mantle cavity for a few seconds, then emerge. Eventually, some miracidia ar seen to swim straight at the snail and enter the pulmonary aperture in order t penetrate into the snail tissue. Though many hours were spent in observing penetratio attempts, at no time were miracidia seen to penetrate through the head, foot, o tentacle of the snail.

The miracidium within the mantle cavity presently applies its apical papilla to th posterior mantle wall and commences to spiral clockwise in wide, circular movements Within a short period, usually not more than five minutes, the apical papilla is com pletely embedded in the snail tissue. At this stage, the snail seems to react violentl to the presence of the miracidium. It spasmodically contracts its body and ma withdraw completely into its shell. In many cases, this snail reaction was sufficie to dislodge the invading miracidium and cause it to swim out of the snail. A fe miracidia, however, persist. In these, the boring movement of the apical papil gives way to a side to side lashing motion of the body which ceases by the end of to 20 minutes. By now, approximately the anterior fifth of the miracidial body h penetrated the mantle tissue.

Miracidial activity at this stage consists of occasional contractions and extension of the body and a constant, vigorous beating of the cilia on its exposed portion Within ten minutes more, the anterior half of the miracidium is embedd edin the snai tissue, next, to be followed rapidly (one to two minutes) by the remaining portion o the body. The complete process of penetration lasts about 40 minutes. Once fully within snail tissue, there are further slow contractions and extensions of the miracidia body and some forward progress within the snail tissue.

To facilitate further observation, snail shell was carefully pried off in one instance to expose the invading miracidium to full view. The snail was then dissected and the piece of tissue containing the miracidium was further studied under the oil immersion objective of a microscope.

All epidermal plates were present, their cilia beating wherever unhampered by snail tissue. Apical gland contents were being slowly but constantly deposited out- side the apical papilla through the ducts. This granular matter could be seen coating the external surface of the apical papilla and dissolving rapidly in the snail tissue around it. There was a distinct area, immediately anterior to the apical papilla, where no snail tissue could be found. This fact suggests the histolytic property of the apical gland secretion and strongly supports the author's case for considering this gland as the main penetration gland of the miracidium.

Observations were carried out for another half hour, during which time about alf the contents of the apical gland were expelled, but no further changes in the iiracidium occurred.

The present author can confirm the observation of some authors, including Dinnik nd Dinnik (1954) that only a very small number of sporocysts develop in the iail in comparison with the large number of miracidia used for its infection. The ndings in the present study would indicate that few miracidia actually ever enetrate the snail irrelevant of the number of miracidia the snail is exposed to, nd this for two reasons:

. A large proportion of the miracidia are attracted to snail excreta, completely disregarding the snail itself.

. The aforementioned snail reaction to miracidial invasion is violent enough to prevent almost all of the invading miracidia from complete penetration.

INFESTATION EXPERIMENTS

Several experiments were performed in order to determine the factors that influence ie development of larval stages within the host snail. Snails of varying ages (i.e. zes), kept at different temperatures, were infected with varying numbers of miracidia uring different months of the year. The results of these experiments showed:

Infection is best achieved when snails are kept individually for eight hours in small (1 × 4 inch) glass vials containing tap water at 24°C and the desired number of miracidia. A small piece of lettuce introduced to each vial aids in keeping the snail relatively sessile, thus assuring better infection rates.

The age or size of the snail seems of little importance in infestation. All snails are equally well infected. However, mortality subsequent to infection is somewhat higher in very young or old snails than in young to medium-aged snails (4–7 mm).

Series performed during October at room temperature (14–27°C) were much less successful (34–52% infection rate) than series performed during August (20–31°C) at room temperature (68–100% infection rate). In addition, development of larval stages (from sporocyst to emerging cercariae) in the snail was slower (47–90 days) in the October series, as compared to the August series (37-79 days).

Infection rate in snails kept at 28°C which were subjected to 10–20 miracidia each was 100%. Other rates were: 96% infection with 5 miracidia, 66% infection with 3 miracidia and 8% infection with one miracidium. However, snail mortality rate was 16% in the 10–20 miracidium group, while no snail deaths occurred with infections of 5 miracidia or less.

SPOROCYST

Developing sporocysts were dissected out of infected snails at fixed intervals an observed in living condition, unstained or stained with vital stains only. Sporocyst in the early stages of development are rather difficult to locate and identify becaus of their minute size. In young snails, however, this difficulty is reduced for the sporo cysts are fairly easily detected within the scant amount of snail tissue.

It has already been pointed out that the invading miracidium, after gaining acces to the mantle cavity, proceeds to penetrate through the mantle wall and progresse further in the snail tissues. The time spent in such travel through the snail hos tissues and the distance traversed, vary widely from miracidium to miracidium While most invading miracidia favour the tissues immediately posterior to the mantl cavity where they become lodged within an hour or so, some are found to penetrat as far as the hepatopancreas and may spend long hours, even days, in reachin their destination. Developing sporocysts were found either free in the body space surrounding the digestive tract or loosely attached to the outer wall of the intestin by a mucoid substance. They were also observed imbedded in mantle tissue, rarel in liver tissue, seldom in the foot or head of the snail. Except for the loss of apica gland contents, no overt changes occur in the miracidium in the course of its migrati through the snail. Thus, when dissected out of the snail at this stage, the miracidiu easily swims away. But once permanently located within the snail tissue, the miracidiu undergoes noticeable bodily changes which justify designating it as a developi sporocyst. Among the initial changes that occur are a decrease in size, sheddi of cilia and epidermal plates and the loss of some internal structures.*

The time lapse between penetration of the miracidium till its transformation int a sporocyst is directly proportional to the distance travelled by the miracidium. I was further noted that appreciable differences in rates of development were manife among various sporocysts, but invariably those located farthest from the mant

---

* Dawes (1960), in studying the penetration of *Fasciola hepatica* and *F. gigantica* into their res pective snail hosts, maintains that it is the young sporocyst and not the miracidium which ente the snail's body. He bases his contention essentially on the fact that the miracidium casts off it epidermal plates just prior or immediately following penetration.

The transformation of the miracidium into sporocyst being a gradual and continuous process it seems to the present author that no clear-cut dividing line can be drawn between the invadin miracidium and the young sporocyst. The point at which the one ceases and the other begins is a best arbitrary. Since numerous morphological and physiological changes are involved, any attemp at selecting one particular morphological or physiological occurrence as a basis of differentiatio can only lead to needless hairsplitting. However, even accepting Dawes' criterion, there would b no grounds for designating the miracidium of *P. microbothrium* as a sporocyst immediately followin penetration since in this case shedding of the epidermal plates occurs only after the larva become permanently located within the snail tissue.

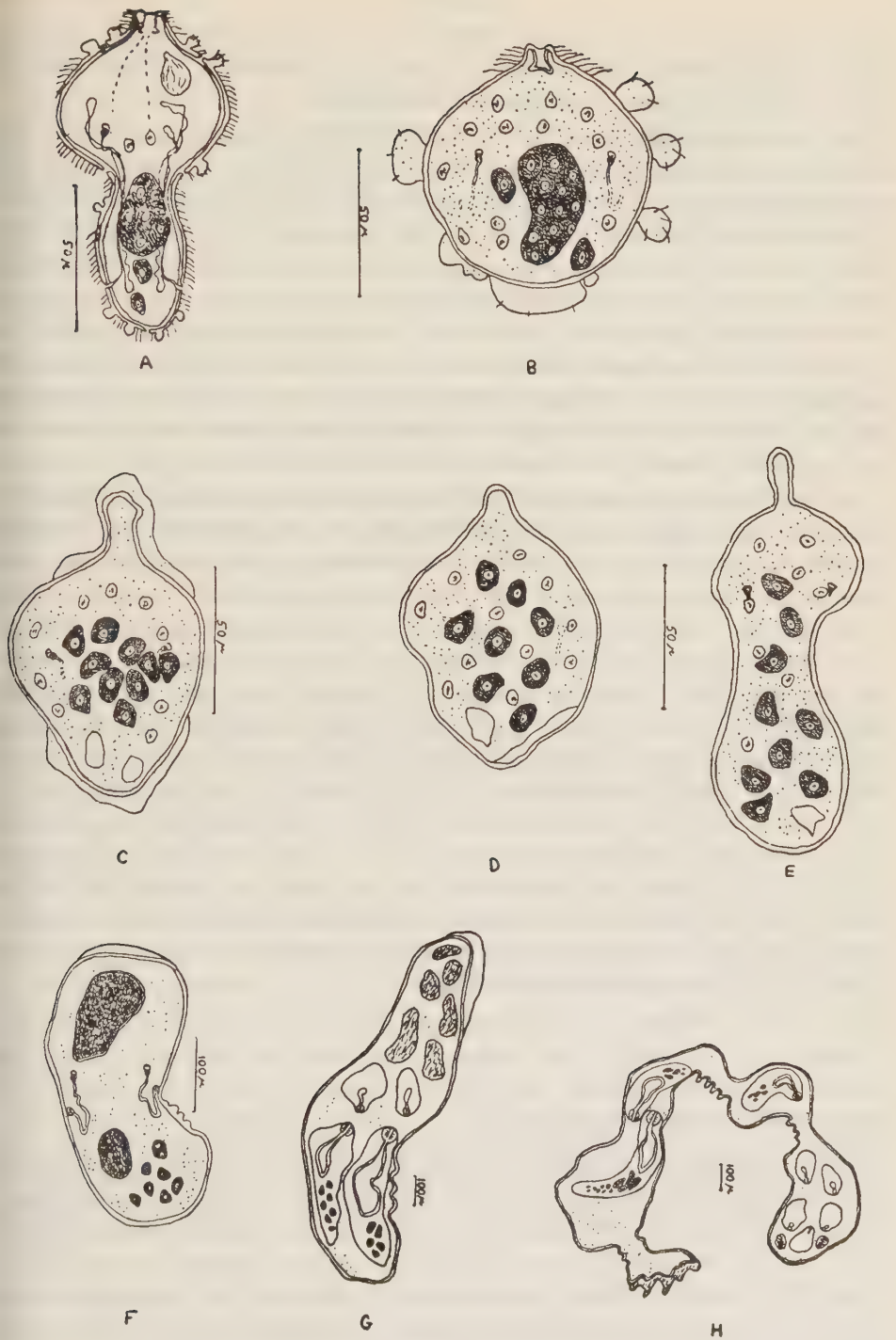


Figure 2
Sporocyst: stages of development in snail host.
2.5–5 hours; B. 5–8 hours; C. 8–12 hours; D. 12–24 hours E. 24 hours;
F. 4 days; G. 8 days; H. 21 days;

cavity were also the most retarded developmentally. The following description c the developing sporocyst is necessarily therefore, a composite picture based o data obtained from numerous separate observations.

After three hours the young sporocyst (Figure 2A) is usually much shorter tha the invading miracidium from which it has evolved. It may measure only 110μ i length and become hour-glass shaped, but it is still capable of periodic contractio and extensions of the body. The apical papilla is completely retracted and the apic gland is an empty, shrunken sac whose outlines can barely be made out. "Penetratio glands"are absent. Other internal structures such as the excretory and genital syste are unchanged in appearance. Superficially, swellings resembling tiny pseudopo appear and disappear sporadically along the surfaces of the epidermal plates. A the epidermal plates are still present but the cilia on some of them, especially o those of the second row, are absent in narrow patches. Remaining cilia, howeve beat vigorously when the live sporocyst is placed in tap water.

After five hours the sporocyst (Figure 2B) is almost spherical in shape, measuri 85 x80μ, with a slight anterior attenuation caused by the retracted apical papill Usually, neither apical gland nor "brain" can be found in the sporocyst. The fla cells may be seen at about the middle of the body but are difficult to make out d to the thickness of the sporocyst at this level. Granulated germinal tissue fills m of the sporocyst body, with the embryo ball or balls as yet visible and unchang The epidermal cells over the middle part of the body are absent or considera shrunk. The cells covering the posterior third of the body are similarly shrunk a bear a few, widely-spaced cilia which give the cells the appearance of minute "prick pears". These shrunken epidermal cells slough off readily at the slightest touch. T first, anteriormost row of epidermal cells, however, is still present, its cells unalter in appearance and completely covered with cilia. In this, the author is in agreeme with Durie (1953) who notes that the cilia are lost from the posterior end first a those of the first set of epidermal plates are shed last. The sporocyst is capable o limited contraction and when placed in tap water manifests forward progress d to the lashing of the remaining anterior cilia.

By the end of the 8th hour the epidermal plates are usually shed (Figure 2C In some cases, however, a few shrunken epidermal cells or traces of such cell especially around the anterior end of the body, may adhere to the surface of th sporocyst for as long as 24 hours.

The next 12 hours in the development of the sporocyst are characterized by th breaking down of the embryo balls into single germinal cells which concentrate i the posterior half of the sporocyst. A few large vacuoles are also noted within th body. Flame cells or their ducts are still difficult to trace, though present. There i some further narrowing at the region of the former apical papilla, giving the sporocys a flask-shaped appearance, but with no essential change in the size of the sporocys (Figure 2D).

hours (Figure 2E)

The sporocyst shows some increase in size, measuring an average $115 \times 75\mu$. A thin ıticle covers the body. The pair of flame cells are easier to observe; they are situated ɛrally in the anterior third of the body. Nuclei of the subepithelium are scattered roughout the body outline, interspersed with germinal cells. The latter are concen- ıted mainly in the central cavity of the body and in a small mass along the posterior ıll of the body.

hours

The sporocyst shows a marked increase in size, becoming saccular and measuring ound $240 \times 110\mu$. A greater number of germinal cells is present, with one or two ıbryonal masses forming anew in the posterior third of the body. The flame cells are ominent, larger than in the miracidium and are situated about a third of the ɔdy length from the anterior end. Their ducts can be traced and are found to be mewhat wider but shorter and less winding than in the miracidium. Each duct ɔens to the exterior through a small vesicle at the middle of the lateral side of the ɔdy.

days

The sporocyst is oval and measures an average $340 \times 100\mu$. It may show two or ore embryonal rediae, the largest, also the most anterior, measuring roughly 100 x $)\mu$ in the germinal cavity of its body. There is a thickening of the cuticle around e anterior and posterior ends of the body and a few wrinkles form in the wall of e sporocyst about its middle.

days (Figure 2F)

The sporocyst averages $390 \times 220\mu$. It is saccular and bent about its center, where slight constriction caused by the contraction of circular musculature often occurs. ıe circular musculature is well developed but longitudinal muscle fibers are not scernible.

days

The sporocyst measures an average $475 \times 250\mu$, with several irregularly spaced ɔnstrictions in its body lending it a compartmented appearance. The sporocyst ɔntains 2–3 developing rediae in the anteriormost compartment and 4–7 embryonal asses in the others. Outlines of a pharynx and gut can be traced, in the developing diae.

days (Figure 2G)

The sporocyst averages $940 \times 270\mu$ and contains 2–3 fully formed rediae bearing sible and functioning flame cells but no birth pore. In addition, 4–5 developing

rediae and 5–8 embryonal masses are present. The fully formed rediae are invariab located in the anterior portion of the sporocyst.

10 *days*

The sporocyst measures an average $1230 \times 310\mu$. It is elongated sausage-lik with deep, transverse constrictions along its length lending it a knobby appearanc and dividing it into two or three compar ments or segments. The 2–3 fully forme rediae occupy the anterior segment while 8–10 developing rediae and numerou embryonal balls fill the rest of the body. First rediae are found free in the snail tissue on the 10 h day.

Mature sporocyst (Figure 2H)

In any discuss on of the mature sporocyst, it is important to bear in mind th extreme variation in developmental rates among various sporocysts.Thus, while som sporocysts commence to liberate rediae by the 10th day and may be found empt by the 16th day, others retain all rediae as late as the 34th day and may still b dissected out of the snail on the 42nd day.

The process of redia-liberation could not be observed under natural condition When the sporocyst was placed under cover slip, however, it was noted that redi tended to leave through a rupture in the sporocyst wall at its anterior extremi

(Anterior-posterior orientation within the sporocyst may, at first glance, see somewhat of a problem, as the sporocyst is bluntly rounded at both ex remities an lacks such characteristic anterior landmarks as a head, pharynx or eye spots. It w be remembered, however, that the excretory system persists relatively unchanged i the sporocyst. Thus, by locating the flame cells and tracing their ducts, the anteri extremity of the sporocyst may be established). Other, less formed rediae are pre vented from escape by the muscular constrictions in the sporocyst body. Whe empty sporocysts are dissected out of infected snails, they are found to be unsegmented saccular, and with a ruptured wall near the anterior extremity. This suggests tha the constrictions of circular musculature delimiting each segment of the sporocys body are relaxed once the rediae within the segment are fully formed, to allow thei escape through the ruptured anterior wall*. As only fully formed rediae are allowe to leave and these never number more than three at any one time, it is obvious tha the liberation process is continuous over a certain period of time. Free rediae were first observed on the 10th day while the earliest empty sporocysts were dissected

* Tandon (1957) observed an obliquely transverse opening at one end of the sporocyst for the emergence of fully developed rediae. A similar structure was seen in some sporocysts in the presen study, but it was more in the nature of a groove rather than an opening and was often situated on the posterior end of the sporocyst. Its function could not be ascertained.

ut on the 15th day. It would seem, therefore, that the sporocyst releases its rediae ntermittently over a period of at least five days.

Bennett (1936) observed that sporocysts reached their maximum size by the time hey started releasing rediae, and that the number of rediae produced by any ne sporocyst never exceeded nine. Dinnik and Dinnik (1954), however, were able o find as many as 28 rediae in one sporocyst. In the present study, the ultimate size f the spo ocyst and the number of rediae developing within it seem to be positively orrelated with its developmental rate. In other words, the longer it takes the sporo- yst to reach the rediae-liberating stage, the larger the sporocyst and the more rediae re produced by it. For example, an unruptured sporocyst dissected out on the 12th ay measured $2350 \times 360\mu$ and contained 16 developing rediae and embryo balls, while a 16th day sporocyst measured $2620 \times 410\mu$ and contained 38 developing ediae and embryo balls. The largest unruptured sporocyst was seen on the 34th ay. It measured $3490 \times 550\mu$ and contained 87 rediae and embryo balls.

Structurally, however, there was no essential difference between the sporocysts f various sizes dissected out from the 19th day onwards.

REDIA

The rediae may be said to start their development from the embryo balls forming within the 48 hour old sporocyst. These embryo balls are not unlike the ones seen n miracidia. (It will be remembered, however, that once miracidia transform into porocysts, their embryo balls are broken down into germinal cells which,by means f cleavage, form new embryo balls in the early sporocyst. It would not be strictly orrect, therefore, to consider the miracidium as already harboring the developing uture rediae.) They are spherical at first but become elongated, measure around 00 x 60μ in the 4-day sporocyst and show the digestive primordia as a fine string f centrally-located cells running the length of the embryo. Development of the igestive system progresses rapidly so that developing rediae in the 5-day sporocyst how distinct outlines of a pharynx and gut. The lumen of the gut, a narrow oesopha- gus, a muscular pharynx and salivary glands are well delimited in developing rediae n a 6-day sporocyst. However, the gut in the now much elongated developing rediae xtends only somewhat posterior to the middle of the body.

The excretory system in the developing redia is at first (5-day sporocyst) represented y two pair of flame cells and their ducts. Two flame cells are situated on each side, ne in the anterior third, another in the posterior third of the body. The fine ducts f the flame cells of each pair meet at about two thirds the body length from the nterior end where they open to the outside through a small common bladder. Later 6–7 day sporocyst), a third, middle flame cell is formed on each side.

A brain mass situated dorsal to the pharynx forms at about the same time as the igestive and excretory systems. Germinal cells form early in the development of the edia and by means of cleavage become embryo balls which number 5–9 at the birth f the redia. Muscular development is the slowest, the developing redia exhibiting

only feeble movements within the 7–day sporocyst. The developing rediae are a first firmly attached to the sporocyst wall, but become free in the central cavity of 7-day sporocyst. By then, they are apparently fully formed but lack a birth pore They remain in the sporocyst for another few days, increasing gradually in size unti they are liberated from the 10-days or more old sporocyst.

At birth, rediae exhibit a marked variation in size and may range from 164 to 264 μ in length and 73 to 116 μ in width. Apparently structural development rather than size is the criterion for the liberation of the rediae from the sporocyst. The free redia is a much more complex organism than the sporocyst. It contains a digestive system a nervous system and germinal primordia.

The young redia (Figure 3) is bluish-white in colour and roughly sausage-shaped

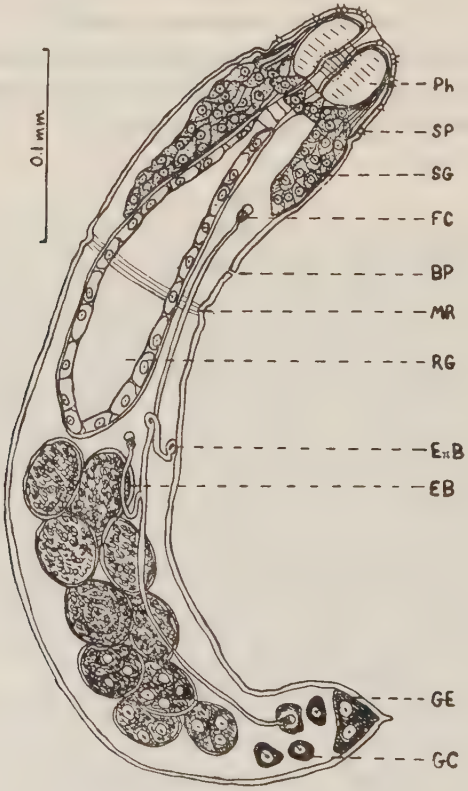


Figure 3
Young redia, lateral view

Most rediae, when teased out of snail tissue and placed in water, are found to curve slightly ventrally. Some, however, may be seen bent sharply inwards to a position where head and tail practically meet. In o hers, movement is limited to a slow side to side twisting of the anterior half of the body or periodic protractions and retractions

of the head region. Young rediae exhibit relatively more vigorous movement than fully mature rediae. Though rediae were observed for long periods at a time, they were never seen to make any appreciable forward progress along the bottom of the dish. Due to the curvature of its body, the redia usually lies on its side in the observation vessel.

The body of the redia is covered with a thin, transparent cuticle which anteriorly folds inwards to line the lumen of the mouth, pharynx and the anterior portion of the oesophagus. Immediately beneath the cuticle are thin layers of cicularr and longitudinal musculature, followed by the epithelial layer of the body cavity. The cuticle around the head region bears a number of minute papillae. In lateral view at least 12, often 14 papillae were counted. They apparently are arranged in symmetrical fashion on all sides of the body (Figure 3). Each bears a short straight hair about 1–2μ long. The papillae are spherical in shape and seem to be in their greater part embedded in the cuticle with just the anterior hair-bearing tip projecting to the outside. Tiny ducts could at times be traced posteriorly from these papillae, but whether the ducts were connected with the brain mass could not be ascertained.

The longitudinal and circular muscle layers in the anterior half of the redial body are more strongly developed than in the posterior half. This fact explains why movements of the body occur mainly in the anterior portion of the redia. Directly behind the level of the pharynx, a band of circular muscle fibers forms a transverse ring in the body wall of the redia. A similar ring of circular muscle fibers occurs at about the level of the middle of the gut. A third ring sometimes is found in the posterior part of the body. These muscle rings are found to function especially when the redia releases its offspring.

The digestive system in the young redia consists of a mouth pharynx, a narrow and short oesophagus and a rhabdocoel gut. The mouth is a short, undistinguished tube that leads into the pharynx. The pharynx is a globular, strongly muscular organ which, when viewed laterally resembles a sagittally sectioned tangerine. It is somewhat wider than long, measuring an average 37μ in length and 40μ in width in the young redia. Occasional contractions of longitudinal and radial muscles in the pharynx cause a transverse extension of the pharynx and a widening of its lumen. At times only the anterior portion of the lumen widens into a small globular hollow. The lumen, when fully expanded, may reach a third of the total pharynx diameter. Circular muscle units in the interior wall of the pharynx form a double row of small dots in lateral view. The pharynx is surrounded by a relatively thick, fibrous memb ane which also envelops the rhabdocoel gut. The pharynx leads posteriorly into a short oesophagus, about 15μ in length and 10μ in width, which opens into the rhabdocoel gut. The gut, a long saccular, roughly flask-shaped organ, is made up of a single layer of large oval to rectangular cells with prominent, eccentric nuclei whose chromatin matter is concentrated in a solid mass in the center. The lumen of the gut stains a bright orange with neutral-red dye; unstained, it appears as a milky, opaque, unsegmented area. Anteriorly, however, where the gut narrows and opens into the

esophagus, it seems to form three transversely rectangular compartments. In young rediae the gut measures 84(62–99)μ in length and 45(26–51)μ at its widest diameter, and extends posteriorly somewhat obliquely to about the middle of the body.

It has been pointed out earlier that the redia is capable of protracting and retracting its head region. It may now be added that the digestive complex (pharynx, oesophagus and rhabdocoel gut) appears at times to move independently within the cavity of the redia. Thus, the pharynx, which normally is situated right behind the anterior extremity, may occasionally be retracted posteriorly a distance of some 10–20μ.

In close juxtaposition with the gut and pharynx are a number of elongated, drop-shaped cells, commonly considered to be salivary glands. The exact number of these cells could not be determined in the living redia as they are closely packed and overlapping one another. Rediae under pressure of cover slip, however, tended to release most of their body contents, salivary glands included, through the birth pore. In this manner, at least 42 such cells were counted in each of several young rediae. When extended, these cells may measure 25μ in length and 5μ in diameter, becoming almost cylindrical in shape. Relaxed, they measure approximately 15μ in length and 19μ at their widest diameter and assume a flask-shaped appearance. Each cell contains a globular nucleus, some 5μ in diameter, whose chromatin matter is in a small mass at the center, the rest of the nucleus remaining clear. The nucleus is situated at the middle of each cell and is surrounded by the granular cell cytoplasm. Anteriorly, the cell narrows into a minute but rather long duct whose opening to the exterior is difficult to trace. There appear to be three groups of these cells. One group is situated dorsal to the pharynx and consists of a closely packed mass of single cells surrounded by a common, thin membrane. Posteriorly, this cell mass extends to about half the length of the gut. Anteriorly, the cells can be traced through their ducts to the vicinity of the mouth, at the anterior margin of the pharynx. A second group of cells, similar to the first but consisting of fewer cells, is located ventral to the pharynx. Because of their smaller number, these cells extend posteriorly only as far as a third of the length of the gut. Anteriorly, however, they terminate at about the same level as those of the first group. The third group consists of single cells which encircle the oesophagus at the level of its posterior extremity and apparently open into the lumen of the pharynx. Aside from the fact that the cells of the third group are not enclosed within a common membranous envelope, they differ from cells of the first two groups in being triangular rather than drop-shaped and having more coarsely granular cytoplasm.

As mentioned previously, the groups of cells just described are generally assumed to be salivary glands. That they are glandular in nature is strongly suggested by their shape, their long ducts and granular cytoplasm. There seems to be some confusion in the literature as to whether one or two types of glands exist. Bennett (1936) and Tandon (1957) describe only one type of gland, while Sewell (1922), Krull and Price (1932) and Durie (1953) distinguish two types. In the present study, two types of glands are indicated: elongated, drop-shaped glands with finely granular cytoplasm

surrounded by a common envelope and single, triangular glands with coarsely granular cytoplasm. Whether both or either type of gland is salivary, histolytic or otherwise secretory in function* and whether the glands open at the mouth, the lumen of the pharynx or, perhaps, the sides of the body (through the cephalic papillae?) are points that cannot be settled satisfactorily at present and bear further investigation.

The nerve centre in the redia is similar to that of the miracidium. Bennett (1936) describes it as a "central fibrous nerve mass" dorsal to the oesophagus, and surrounded by nerve cells. The latter are characterized by nuclei that stain darker than other surrounding nuclei. In the present study, the nerve centre was seen best in the developing redia, prior to liberation, where it consisted of a roughly oval, fibrous mass, dorsal and adjacent to the oesophagus and posterior margin of the pharynx. The nerve cells, evident by means of their deeply staining nuclei, surround the posterior margin of the brain mass. No nerves could be traced. In the free redia, the brain was difficult to locate since it was, in its greater part, obscured by the dorsal "salivary" glands which were superimposed over it. It measured about $30 \times 20\mu$.

The excretory system in the free redia is essentially the same as in the developing one described earlier. The flame cells measure about 15μ in length and 6μ in diameter. To properly trace the excretory system, the redia has to be viewed either from its ventral or dorsal surface. Two flame cells are situated laterally, one on each side of the gut, about $^1/_5$ the body length from the anterior end. A similar pair of flame cells is situated roughly $^1/_6$ the length of the body from the posterior end. A third pair of flame cells is located somewhat posterior to the middle of the body. The ducts from the three flame cells on each side of the body unite at about the middle of the body whence they proceed through a short common duct to empty at a small excretory bladder which opens on the side of the body, somewhat behind its middle.

The genital system in the redia is represented by germinal epithelium, germinal cells and embryo balls. Germinal epithelium, located at the posterior end of the germinal cavity, produces germ cells which develop into embryo balls. In newly emerged rediae, there are between 5–9 embryo balls, the largest of which, also the most anterior, measuring about 30μ in diameter. The embryo balls and germ cells occupy roughly the posterior half of the body in young rediae.

Newly emerged rediae lack a birth pore. This structure usually develops within a day or two after liberation, irrespective of the size of the redia. Bearing in mind that the size of the redia is variable at birth, it is not unusual to find a birth pore in a redia measuring 240μ in length while one measuring 255μ in length may lack this structure. In young rediae the birth pore is a small, almost indistinguishable break in the cuticle on the ventral side of the body. It is surrounded and marked by a few wrinkles in the cuticle, but remains closed and flat till the maturation of

* Kruidenier (1953a, 1953b) described mucoid glands in distomate cercariae some of which resemble the above-described redial glands in arrangement and morphology.

the redia. In he gravid redia, about to release offspring, the birth pore is a prominent structure and is situated at the center of a small, well-muscled protuberance, some 20–40μ wide, on the ventral side of the body. In different rediae, it may be found anywhere from $^1/_3$ to $^1/_6$ the body length from the anterior end. Though the birth pore is well-muscled it does not seem to play an active role in the release of offspring, but simply opens when pressure inside the redial body is increased due to contractions of the circular muscle bands.

Like the sporocyst, the redia appears to reach maximum size just prior to the release of offspring, some 10 days after liberation from the sporocyst. It may then measure an average 967(720–1173)μ in length and 206(125–266)μ at its widest diameter. Structurally and morphologically the mature redia differs only sligh ly from the young redia, the main points of difference being in the size and extent of the digestive system, the number of embryo balls, and the colour of the body. The pharynx in the mature redia measures an average 47(40–59)μ in length and 52(44-59)μ in width. The rhabdocoel gut, while almost doubling in size (164(119–239)μ in leng'h and 76(59–92)μ in width), at best extends only $^1/_6$ the length of the body from the anterior end. The reason for this lies part'y in the marked increase in length of the redial body and partly in the fact that the rapid growth in size and number of the embryo balls filling up all available space within the redia causes a marked displacement of the gut along a dorso-ventral p'ane, the gut at times appearing to lie on top of or under the pha ynx rather than behind it.

The young redia contained between 5 and 9 embryo balls which occupied the posterior half of its body. The mature redia may contain up to 27 embryo balls and developing offspring which fill most of its germinal cavity. These offspring of the redia, as will be shown presently, comprise cercariae and daughter rediae in alternate succession. Daughter-redia production constitutes an important and essential phase in the life-cycle of the species.

The excretory system is essentially unchanged except for the ducts along the sides of the body which lengthen in proportion to the increased body length and the excretory bladders which open at just about the middle of the body.

The body of the mature redia retains the "sausage" shape but may show several transverse low ridges along its length and a small nipple-like attenuation at the tip of the tail end. Finally, the mature redia is milky-white in colour and is less translucent than the young redia.

Bennett (1936) states that rediae begin feeding upon the snail tissues immediately after release and migrate slowly into the liver and ovo-testis where they complete their development. In the present study, the author was unable to establish the presence of snail tissue ihe th redial gut. However, the presence of a well-developed digestive system in the redia, coupled with the higher mortality, the greatly diminished reproductive capacity and stunted growth of infested snails, indirectly suggests that rediae may feed on snail tissue. When the shell matter of an infested snail is carefully removed, no immediate external damage to the snail tissue is apparent, the hepato-

pancreas, ovo-testis, and mantle tissue remaining superficially intact even while filled with rediae and immature cercariae. Whether the damage to the snail was caused by rediae, immature cercariae or both, and the extent of such damage, could not be ascertained, but it is obvious that the snail must possess remarkable recuperative powers to be able to survive such continuous, massive infestation.

Both mature and young rediae were found in the body spaces surrounding the digestive tract or within the hepato-pancreas, ovo-testis and mantle tissue. The presence of mature rediae in mantle tissue and body spaces surrounding the digestive tract, the preferred loci for sporocyst development, contradicts the contention that all rediae migrate into the liver or ovo-testis to complete their development.

Pedogenesis of the rediae

In an attempt to follow pedogenesis in the redia, a minimum of three infected snails was dissected daily, from the 10th day of infestation till the 40th day. Thereafter, a single snail was dissected at five-day intervals till the 90th day of infestation. Several additional snails were dissected over a period of seven months from the date of infestion. Each of the snails used in these studies was initially exposed to 20 miracidia. Rediae teased out of the snail tissue were measured, then studied closely under microscope to determine the number and nature of developing offspring. In this manner, it was possible to construct the following composite picture of pedogenesis in the redia:

10th day* First rediae are liberated from sporocyst, and contain 5–9 embryo balls.

16th day Rediae** markedly increased in size, and contain 1–2 developing daughter rediae and up to 15 embryo balls. The developing daughter rediae are always situated anterior to the embryo balls and are readily identified by the characteristic redial pharynx and elongated body.

20th day Rediae contain 1–2 fully-formed daughter rediae, 1–2 developing cercariae and 25 embryo balls. The daughter rediae are in all respects identical to sporocyst-born rediae and are situated anterior to the developing cercariae which, in turn, are anterior to the embryo balls.

23rd day Rediae contain no daughter rediae but 2–5 developing cercariae and 27 embryo balls.

* The dates given refer to the number of days which elapsed from infestion of the snail with miracidia, not the age of the redia. They also are meant to represent the minimal number of days within which a particular stage of development in the redia is reached.

** The term "rediae" is arbitrarily taken to mean the redia or rediae most advanced developmentally at the particular day of observation.

26th day Immature cercariae are found free in the hepato-pancreas of snail.

40th day Rediae are seen containing two anteriorly developing cercariae, followed posteriorly by one developing daughter redia and two embryo balls. The developing cercariae may be distinguished from the developing daughter rediae by: a. the lack of a pharynx, b. more oval and less elongate than the rediae, c. brownish in colour as compared to the whitish-grey rediae, d. usually arranged transversely in the body of the parent while daughter rediae are arranged longitudinally. The fact that only two embryo balls are present suggests that the germinal epithelium is exhausted and the mother redia must be aged.

45th day First dead redia seen. Superficially, it resembles a sporocyst, the body appearing knobbed or compartmented, the cuticle wrinkled and thin. It is a dirty-grey in colour, seems opaque, and contains only one formed daughter redia, no embryo balls, but a few germinal cells. Its length, 530μ, suggests that apparently rediae shrink considerably in size toward senescence.

After 50 days Dead rediae, containing 1–2 daughter rediae or 1–2 immature cercariae with short tail stems, are found regularly but never more than a few in any one snail.

80th day A dissected snail contains 347 rediae at all the stages of development but with young rediae predominating. A large number of developing cercariae is al o present.

90th day A dissected snail contains 762 rediae at all the stages of development in addition to cercariae.

3–7 months Dissected snails contain hundreds of rediae plus many cercariae.

216th day Last infected snail dies, shedding cercariae to the end.

As outlined above, pedogenesis in the redia of *P. microbothrium* is in close agreement with that described by Dinnik and Dinnik (1954). In the latter study, however, the rate of development of the various redial stages was appreciably slower, due, probably, to the fact that infested snails were kept at a temperature of 18–22°C.

Dinnik and Dinnik maintained that they "were able to trace quite definitely four redial generations which develop within the first 80 days after penetration of miracidia into the snail" Thereafter, the overlapping of the different generations of rediae within the snail prevented further distinction between the various generations. The conclusions of Dinnik and Dinnik were based on the fact that three stages of mature rediae were seen within the snail at the same time:

a. Aged rediae containing few daughter rediae only — designated as first generation rediae.

). Mature rediae containing cercariae only — designated as second-generation rediae.

. Mature rediae startin to form daughter rediae — designated as third-generation rediae which contain fourth-generation rediae (the daughter rediae).

The author, while agreeing in principle with Dinnik and Dinnik as to the likelihood of successive redial generations within the snail, does not, however, share their certainty as to the number of redial generations that may be discerned. In interpreting the data, Dinnik and Dinnik fail to take into account the fact that sporocysts constantly liberate rediae over a period of at least 20 days and that therefore the following possibilities exist:

. : That a young sporocyst-born redia may be mistaken for a young daughter redia, as the two are morphologically indistinguishable.

. : That the aging sporocyst may release rediae which produce cercariae only.

. : That not all rediae mature in the same length of time nor do they reach senescence at the same rate and therefore no ordered or synchronized succession of redial generations may occur.

In the present study, the overlapping of redial generations offered a serious obtacle in interpretation of the data as early as the 30th day after infestation of the nail with miracidia. To overcome this difficulty several attempts at transplanting ingle rediae from an infested snail to an uninfested one were made, but these proved nsuccessful, the transp anted rediae dying quickly within the new host. It would ppear, therefore, that any interpretation of pedogenesis in the redia is of necessity peculative in nature and, at best, based on indirect evidence only.

That there are many successive redial generations in the life-history of *P. microothrium* is strongly suggested by the fact that infested snails, dissected as late as 7 nonths after infestation, contained many rediae at all stages of development*. Theoretially, postulating a 35 day limit for the productivity of the sporocyt and a 50 ay limit for the life span of the redia, it is obvious that a succession of at least four edial generations is necessary to account for the presence of rediae in the snail 210 ays after infestation. On the basis of available data, it seems fairly clear that sporoyst-born rediae, or, borrowing Dinnik and Dinnik's terminology, "first generation ediae", initially produce 1–2 daughter rediae, next produce about 20–25 cercariae, nd finally yield a few more daughter rediae. What happens in succeeding redial enerations is less certain. While it is reasonable to assume a similar cycle in at least ome daughter rediae, it is very unlikely that all rediae of succeeding redial geneations produce daughter rediae. This for a simple reason: if every redia in succeeding

* Dinnik and Dinnik (1954) found rediae in a snail 504 days after infection with miracidia of *'.microbothrium*.

redial generations were to produce even as few as two daughter rediae, the number of rediae within the infested snail would increase on the order of a geometric progression, easily exceeding a thousand after a few short months. This was never seen to happen. Snails harbored only hundreds of rediae, never thousands. Therefore, either rediae of succeeding redial generations produce only one daughter redia apiece or, what is more feasible, only few rediae in each generation produce daughter rediae.

CERCARIA

The sporocyst-born redia contains 5–9 embryo balls at birth. As only 1–2 of these develop into daughter rediae, it is reasonable to assume that the remaining embryo balls are the future cercariae. As embryo balls, they measure about 30 x 30μ and are arranged roughly two abreast within the posterior third of the redia. Development occurs slowly, the more anterior embryo balls gradually becoming oval, increasing in size, and arranging in tandem fashion with their extremities pointing toward the sides of the redial body.

Rediae on the 20th day of infestation usually contain 1–2 developing cercariae measuring an average $120 \times 80\mu$ in which digestive and excretory primordia may be discerned. The digestive primordium consists of a group of centrally-located cells which form a figure-eight pattern in the anterior end of the developing cercaria. Of the two lumina thus formed, the anterior one represents that of the future oral sucker, the posterior one, that of the rhabdocoel gut. The excretory system is represented by two fine, straight ducts, one on each side fo the body, which extend anteriorly to about the middle of the body but open separately at the posterior end. No flame cells can be traced at this early stage.

The developing cercaria manifests rapid development within the next few days. The posterior tip of the body shows a transverse groove both on the dorsal and ventral surface, the constricted part being much wider than long and developing quickly into a tail stem. The two lateral body excretory ducts now unite in the posterior end of the body into a single medial duct which leads posteriorly, slightly coiled, to empty at a small medially-located and dorsal excretory pore. The excretory pore is just anterior to the line demarcating the tail stem from the body. Leading posteriorly from the pore, a thick, coiled caudal duct winds its way at the center of the tail stem to bifurcate at the end of the tail and open in two ducts at its sides. Meanwhile, the rhabdocoel gut bifurcates, the resulting caeca extending laterally and somewhat posteriorly to form a small inverse "U". The oral sucker is joined posteriorly to the gut through a short and narrow oesophagus. A mouth opening forms at the anterior end.

The genital primordium is represented by a small mass of deeply staining cells situated immediately posterior to the bifurcation of the gut.

By the 23rd day of infestation, there are usually 2–3 developing cercariae within the redia, ready to be liberated. These are always situated anterior to less developed cercariae and in some rediae may be separated from them by a transverse con-

triction in the redial body. Like the redia, the developing cercaria shows great variability in size at birth. Its body may range from 203 to 270μ in length and 132 to 134μ in width while the tail stem varies between 55–73μ in length but is always wider than long. Just prior to its birth on the 25th day of infestation, the developing cercaria may show a pair of eye spots developing in the anterior third of the body. A few flame cells may also be detected in some developing cercariae prior to birth.

Immature cercariae are liberated by rediae in the same manner as daughter rediae and are capable of limited contraction and extension of the body. They slowly migrate to the hepato-pancreas and ovo-testis of the snail, where they complete their development.

Shortly after liberation, the immature cercaria shows an excretory system similar to that of the mature cercaria but without the characteristic refractory granules ("excretory concrements") in the excretory ducts. The mouth opening and oral sucker are well formed and the oesophagus opens into short caeca extending not quite a third the body length from the anterior end. The eye spots are prominent, with pigment surrounding each lens in a transversly oval mass. The body is lined with a very thin cuticle and has a few large, spherical to hexagonal cystogenous cells which are as yet devoid of the characteristic rods. Muscle layers are not discernible.

Within the next few days, the immature cercaria gradually increases in size, the tail becoming longer than wide. The digestive system features a longer oesophagus and prominent, thick caeca which extend laterally and posteriorly to about the middle of the body. The excretory system remains unchanged except for a gradual anterior displacement of the excretory pore which comes to lie dorsal and just posterior to the union of the lateral ducts. Eventually a small excretory bladder forms around the pore. The caudal excretory duct is now straight and terminates in a small bladder not quite reaching the end of the tail. Two minute ducts lead from this bladder to open at the sides of the tail. Numerous flame cells may be detected in the body but their capillaries cannot be traced. Pigment is still massed around the eye spots but it is starting to branch out in small finger-like projections under the surface of the cercaria. The cystogenous cells are more numerous but are found only posterior to the eye spots.

An acetabulum forms in the immature cercaria shortly after birth but can be seen with difficulty only. In a cercaria measuring 302μ in body length, it may be seen clearly as a subterminal, ventral sucker, somewhat wider than long but not strongly muscular as yet. By now the digestive system is like that of the mature cercaria, the oral sucker strongly developed and lying directly under the cuticle at the anterior extremity of the body, while the gut caeca extend to about the anterior margin of the acetabulum.

The only change in the excretory system is a further displacement of the excretory pore and bladder to a position just anterior and dorsal to the point of union of the lateral excretory ducts. The eye spots are very prominent and dark, the pigment around them scattered in small, irregular blotches. Cystogenous cells still devoid of

rods fill the whole body except for a small area around the mouth and anterior 1/ of the oral sucker, and make the body opaque. The cuticle surrounding the bod is thicker and muscle layers are discernible.

In an immature cercaria measuring 420μ in length and having a tail 450μ long cystogenous rods make their appearance. These are packed in parallel bundles withi the cystogenous cells and fill the outline of the entire body except for the anterio tip which is left free of rods. Outlines of the cystogenous cells cannot be traced becaus of the abundance of rods within them. At the same time, refractory granules appea in the lateral excretory ducts but are, on the whole, somewhat smaller than in th mature cercaria. The eye lenses are very prominent and surrounded by black cone of pigment. Pigment fingers originating from them now extend posteriorly and laterall ly under the surface of the body to about half its length. The acetabulum is wel developed and in position and size the same as in the mature cercaria. A genital atriu can now be discerned on the ventral surface of the body, medially-located, just post erior to the bifurcation of the intestinal caeca. The genital pr mordia are grouped i two small cell masses, one surrounding the genital atrium, the other surrounding the excretory bladder.

In a cercaria measuring 437μ in length and 343μ in width, with a tail 468 x 93 μ, pi ment covers the whole body except for small clear areas around the mouth a genital atrium. When dissected out of an infected snail and placed in water, such cercaria may swim around freely but, unlike the mature cercaria, is incapable successful encystation. Apparently, the ability to encyst is reserved for escap cercaria only.

Cercariae begin emerging from infested snails 37 days after exposure of the sna to miracidia, the cercariae averaging 15 days for initial development within the red and an additional 11 days to reach maturity in the hepato-pancreas of the snail.

Mature Cercaria

The mature cercaria is a large, vigorously swimming organism, clearly visible to th naked eye due to the heavy, brown pigmentation which spreads over the whole surfac except the head region and a small area around the genital atrium. When the cercari is slowed down by the addition of a few drops of chloroform or methyl cellulos in water solution, its body is an average 380(203–532)μ long and 289 (172–375)μ wide while the tail is 750 (674–814)μ long and 84 (70–94)μ wide.

When relaxed and straightened the cercarial body is roughly pear-shaped, it maximum width somewhat in its posterior part, with a cone-shaped anterior extremit (Figure 4). Frequently its anterior extremity is folded underneath the body, lendin the cercarial body a transversly oval or circular appearance (Figure 7c). The tail i contractile and generally about twice as long as the body. In lateral view, the outlin of the cercaria appears banana-shaped, the ventral surface being concave and deepl grooved, the dorsal surface sharply convex. Pigment granules are widely distribute

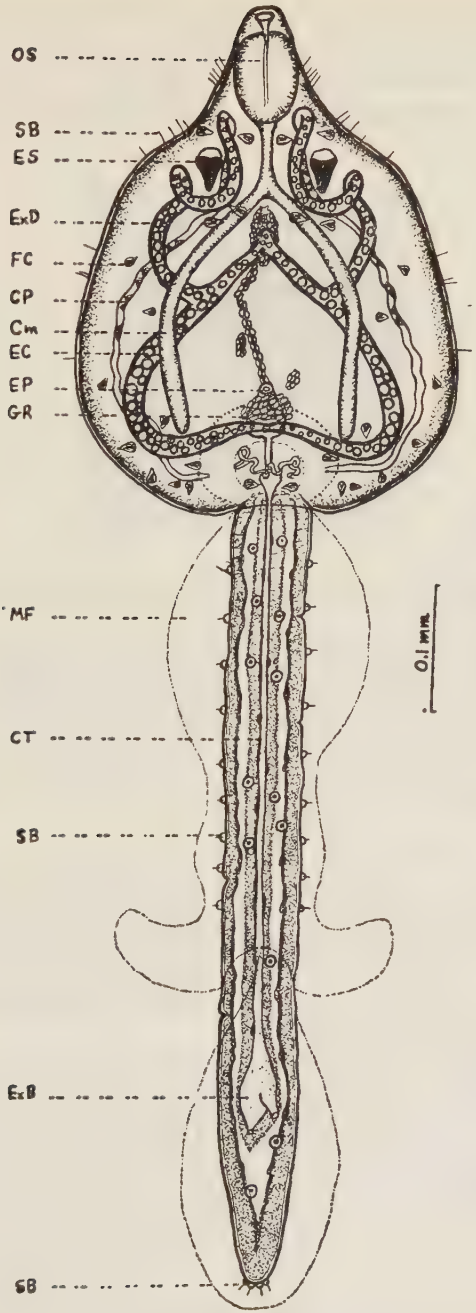


Figure 4
Cercaria: general (dorsal) view.

over the body mainly along a series of fine rows that extend under the surface of t body from mouth to acetabulum (Figure 5). There are, however, three unpigment areas in dorsal view: The first is about $^1/_3$ the length of the body from the anteri end and corresponds to the region of the genital atrium; the second, roughly

Figure 5
Cercaria: microphotograph showing arrangement of pigment along longitudinal rows

the length of the body from the posterior end, is situated around the opening of excretory bladder. The third clear area is around the oral sucker.

A fine, narrow groove extends on the dorsal surface of the cercaria from the cretory pore to the attachment point of the tail. The ventral surface of the cercar body shows a deep groove along the whole length and bears the subterminal a tabulum.

The body is covered with a thin cuticle, about 3μ thick. The cuticle around t mouth and oral sucker is somewhat thinner than that around the rest of the boc Within the cuticle, running along the inner surface, several longitudinal lines sugg ting structures such as muscle fibers may be seen. These, however, disappear as cuticle expands under continuous cover slip pressure, indicating that they are cuticu folds rather than histological structures.

The mature cercaria is opaque not only because of the pigment but also becau of a dense mass of short, refractory, cystogenous rods. These rods measure 11 x and are distributed in a somewhat disorderly fashion, crowding all available spac within the parenchyma of the body. Common fixatives, such as Bouin fluid, do n destroy or damage them. They become invisible, however, in glacial acetic acid.

The mature cercaria has a pair of eyes which, in the extended body, are locat just under the cuticle on the dorsal surface, about 125μ behind the anterior extremi They are conical in shape, 72μ apart, and consist of a black pigment mass posterio and a clear refractile lens anteriorly. The eyes lie with their lenses pointing forwa and upward while the points of the pigment masses are directed posteriorly a ventrally. They average 27μ in length and 20μ in base diameter. The lens is about 1

thick and surmounts the base of each eye. In cases where the body is shrunk and its cone-shaped anterior extremity is folded underneath it, the eye lenses are seen protruding from the outlines of the body (Figure 7c).

The acetabulum is mounted posteriorly and subterminally on the ventral surface of the body. In the relaxed condition in living cercariae it is ellipsoid, measuring 114(90–138)μ in width and 83(75–91)μ in length, with a lumen diameter of 34(29–47)μ.

Tail appendages

The tail is cylindroid, about 750μ long when extended and around 84μ wide at the base. It tapers gradually towards the posterior end. It consists of an outer sheath of large muscular cells enclosing a cavity filled with fluid within which the caudal excretory canal runs straight and unattached.

A striking feature of the cercaria is the presence, on and around the tail, of a peculiar-fin-like gelatinous envelope. This structure is quite invisible in unstained cercariae swimming in water or in those stained with vital stains only, and it is therefore not surprising that previous investigators did not notice it. Furthermore, this structure does not show in specimens fixed in Bouin and stained by alum carmine. Its presence was suspected after close observation of the free-swimming cercariae in a Syracuse dish. When, subsequently, the cercariae were placed in a suspension of India ink, the envelope-fin became visible.The results are shown in Figure 6. A 0.2% solution of gentian violet produced an effect similar to that achieved with India ink, the dye particles adsorbing to the surface of the envelope-fin. Later investigations showed that a 0.1% toluidine blue in 1% tartaric acid solution stains the tail tissue while the gelatinous fin stains a deep pink color, which remains fast for several hours after removal of the cercaria from the stain. The envelope-fin also became visible by refraction in bovine serum warmed to 30°C.

A series of histochemical tests were performed on the cercaria in an attempt to determine the chemical composition of the envelope-fin*. The latter was found to be strongly acidic, probably sulphated mucopolysaccharide, with little or no protein. Minute droplets of lipids could be observed dispersed in the substance of the envelope-fin. The whole structure was apparently surrounded by a very thin lipoid pellicle. The term "mucoid fin" was consequently adopted for this structure.

Studies of immature cercariae dissected out of the snail host showed that the mucoid fin does not develop until the cercariae are about ready to emerge. In the mature cercaria the mucoid fin may be divided into three distinct sections (Figure 7):

1. an anterior semi-cylindrical section;
2. a middle trapeze-shaped section;
3. a posterior fin-like section.

The anterior section is attached to the ventral surface of the tail, its hind portion partially wrapping the sides of the tail. It originates at a point about 50μ from the

* The author wishes to express his appreciation to Prof. M. Wolman, of the Pathology Dept. for his help in the histochemical investigations.

Figure 6
Cercaria: microphotograph showing outlines of the mucoid fin in India ink suspension

base of the tail. When observed from the ventral side it first flares out laterally and posteriorly, measuring 190μ at its widest part, then narrows again. This section measures approximately 350 μ in overall length.

The middle section resembles a trapeze with the base directed anteriorly. It wraps around the tail and measures 197μ at its widest point and 80μ in length.

The final section is a thin, membrane-like structure that lies in a dorso-ventral plane perpendicular to the body of the cercaria. It extends posteriorly to about 30μ past the tip of the tail. When the cercaria is under cover-slip the final section is compressed dorso-ventrally and appears to lie in a horizontal plane. It then measures 130μ across at its center.

The mucoid fin as a whole, though constant in shape, is very elastic and forms occasional folds and wrinkles during the movement of the tail.

Sensory bristles

Sensory bristles have been described in several species of cercaria. Gordon, Davey

nd Peaston (1934), Vercammen-Grandjean (1951) and Fain (1952, 1953) described iem in schistosome cercariae. Baugh (1954) noted such bristles on a new furcocercous ercaria of the Vivax type. Ullman (1954) saw them in pleurolophocercous cercariae.

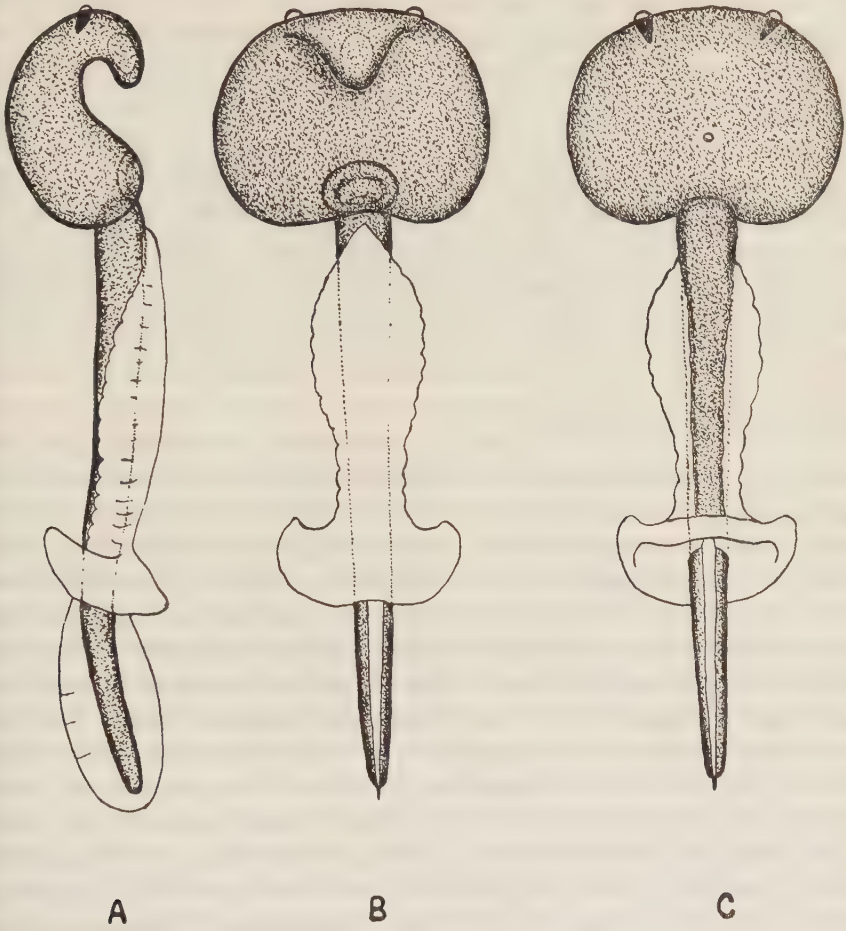


Figure 7
Cercaria: schematic representation of the mucoid fin on tail
A. Lateral view; B. Ventral view; C. Dorsal view.

To the extent of the present author's knowledge, no such bristles have been reported n paramphistome cercariae.

Very fine bristles were found at several loci on the body and tail of the present ercaria. On the body, they were grouped mainly around the anterior region. None vere found on the posterior third of the body. Due to the heavy pigmentation of he cercaria, only bristles emerging from the margin of the cuticle could be seen. These varied in length from 15 to 25μ. Some differences in thickness of bristles were lso noted. The greatest number of bristles counted on any one individual was 36.

They were distributed in an almost symmetrical pattern. An exact count was difficu to obtain because of constant movements of the cercaria. At the same time, tl bristles were best seen on a slightly moving cercaria for then they would move count to the movement of the body and stood out better. A phase microscope w found superior to an ordinary one in observation of sensory bristles.

Four distinct sensory bristles, each arising from a small papilla, are located c the extreme tip of the tail. These were seen on each of at least fifty cercariae examine They average 15μ in length and may be seen even after the death of the cercaria. some specimens, tiny nerve fibers could be seen entering the bristle-bearing papilla In addition to the four bristles just described, two series of short setae, surmounte on evenly spaced, separate papillae, extend at the sides of the tail along the anteri two thirds of its length. The observed arrangement of bristles on the tail and boo of the cercaria is shown in Figure 4. This drawing is a composite picture based o observation of about 50 cercariae.

Internal anatomy of the cercaria

The digestive system in the mature cercaria consists of a mouth, oral sucker, oes phagus and a pair of intestinal caeca. The oral sucker is teminal and oval. It m sures 58.5(44–62) μ in width and 75 (47–110) μ in length. It is situated a short dista posterior to the mouth opening, and is easily visible when the cone-shaped ante extremity of the body is fully extended. The mouth opening is surrounded by min papillomate prominences of the cuticle, apparently eight in number. Within the o sucker there are two bundles of small, grape-like glands, located laterally and opp site each other, just posterior to the mouth opening. These gland bundles may be se only for seconds at a time when the mouth opening is viewed en face. Each bundle w estimated at 10 μ in width and was composed of at least five separate cells. It may presumed that these glands function during the encystation process of the cercaria

The oesophagus and intestinal caeca can hardly be observed in the living cercaria b they can be seen just prior to its death under pressure of cover slip. The oesophag is narrow, about 40 μ in length, and bifurcates just posterior to the eyes to form t intestinal caeca. A slight thickening of the oesophagus at the bifurcation point len it a bulbous appearance. The caeca are thin, 15 μ wide, pass parallel to both side ma gins of the cercarial body and terminate at about its posterior extremity, almo reaching the acetabulum.

The reproductive system of the mature cercaria is represented by a tight cluster of deeply staining, oval cells which lies medially, just anterior to the acetabulum a dorsal to the excretory bladder. It measures roughly 50 $\times$ 30μ and is presumably t ovarial primordium. A similar dense mass of cells, measuring 60 $\times$ 25 μ, is locate anterior to the former and just posterior to the bifurcation of the intestinal caeca, a is probably the primordium of the future genital atr um. A thin string of oval cel representing the uterine primordium, connects the anterior and posterior genital p

mordia. Two rudimentary testes lie diagonally in the central field of the body, one on each side of the primordial uterus. They measure approximately $15 \times 10 \mu$ each. These genital primordia are best seen in fixed material where they appear as aggregates of small oval cells, each cell measuring about $6 \times 4 \mu$. In living, unstained cercariae a clear, unpigmented area appears on the dorsal surface just posterior to the intestinal bifurcation. It is roughly ellipsoidal and measures 25μ across. On the ventral surface at this spot, o such clear zone appears, but instead, a definite opening may be discerned in fixed specimens. The small pore is surrounded by cells belonging to the genital primordia and is probably the opening of the future genital atrium.

The excretory system consists of a group of flame cells, ascending and descending excretory ducts, a caudal excretory tube and an excretory bladder.

The excretory bladder is roughly conical in shape, measuring 22.5 (18–29) μ in base diameter and 28.5 (18–33) μ n length, and is seen best in fixed and stained preparations. It is located mesially, dorsal to the acetabulum, with the base lying immediately above the anterior margin of the acetabulum and the apex poin ing anteriorly. It opens on the dorsal surface through the excretory pore which appears as a disc measuring approximately 9μ in diameter. In fixed specimens the excretory pore may be displaced and appear to open not at the apex of the excretory bladder but at its base or lateral to it.

Two thin ascending excretory ducts, about 5μ in diameter, originate at the lateral margins of the acetabulum, pass outwards and bend forward, curving gradually till about the level of the oral sucker. Then they loop sharply back, increasing appreciably in diameter, and return posteriorly and laterally in a wide arc in the space between the eyes, as descending excretory ducts. At a point lateral to the apex of the eye, the descending ducts form a tight loop and continue posteriorly and inwardly till the middle of the body where they pass ventral to and between the intestinal caeca and bifurcate. One branch from each duct passes anteriorly and mesially, the two branches meeting just posterior to the primordium of the genital atrium. The anastomosis thus formed bears a slight diverticulum at its medial point. The posterior branches of the bifurcation turn outwards and continue posteriorly to about the level of the anterior margin of the acetablum, where they turn sharply inwards and terminate at the base of the excretory bladder, thus forming a pattern of an inverted heart occupying two thirds of the cercarial body.

Seven ciliary patches were detected within each of the ascending excretory ducts, four near the middle of the body, the other three in the vicinity of the eyes. The beating of the cilia in each patch was directed anteriorly, which suggests that the direction of flow within the ducts follows the course of the excretory ducts as described above.*

The descending excretory ducts range from 3 to 25μ in diameter and are filled with refractive globules of varying diameters. The larger globules, up to 15μ in diameter, are located in the central portions of the descending ducts, while the smaller

* Ciliary patches are noted in paramphistome cercariae by Sewell (1922) and Porter (1938), but the direction of the ciliary beat is not stated.

ones, around 3 μ in diameter, are limited to the extremities of these ducts. The name "excretory concrements" is proposed for these globules and "concrement ducts" for the above described descending part of the excretory system. It occurs in several known species of paramphistomid cercariae, as well as in some other cercariae.

The caudal excretory tube is a thin, straight vessel running along the axis of the tail. It apparently joins the excretory bladder through an "islet" aperture similar to those described for some furcocercous cercariae. Its anterior extremity is located dorsal to the lumen of the acteabulum and is surrounded by a fine network of capillaries, the connection between them and the excretory system being uncertain. The caudal excretory tube measures 5 μ in diameter and passes along the center of the tail cavity, enclosed in a cellular sheath, 45 μ wide. It moves loosely within the fluid filling the cavity of the tail. The tube terminates about 150 μ from the end of the tail in an oval bladder 32 μ in width. This bladder opens through two fine ducts at the opposite lateral surfaces of the end of the tail. The openings are minute and can be seen only on rare occasions.

The flame cell pattern in the mature cercaria is extremely difficult, often impossible to determine, due to the opacity and heavy pigmentation of the organism.

Fourteen pairs of flame cells were detected in immature cercariae, dissected out of the host snail. Four were located near and around the eyes, two toward the middle of the body and eight pairs in the vicinity of the acetabulum. Their arrangement is shown in Figure 8. Of the fourteen pairs of flame cells, only six could be identified after many hours of observation in the mature cercaria*. Here they were best seen just prior to the death of the cercaria, while under pressure of cover slip. No capillaries of flame cells could be traced.

Activity of the cercaria

When freely swimming, the cercaria advances due to lashing and rapid vibrations of the tail which pull the body along. The mucoid fin apparently plays an important part in the propulsion mechanism of the cercaria. In a Syracuse dish, the cercaria shows preference for the bottom of the container. After moving about for 18(8–40) seconds the cercaria comes to rest on the bottom of the dish. It may rest in several positions. It may curl up on its ventral side by use of oral sucker and acetabulum, while the tail hangs motionless at a 45° angle from the horizontal. At other times it will lie on its side. Frequently, it will come to rest on its dorsal side with both oral sucker and acetabulum extending upwards and inwards till both ends meet; then the cercaria straightens out and rolls over on its side. After a res period of about 5(4–6) seconds, the tail starts its typical lashing vibration and seconds later, the cercaria is off again.

A definite thigmotaxis is exhibited, the cercaria rarely coming to rest on the surface but mostly moving about the bottom of the dish. Its swimming direction is random

* Bennett (1936) reported 16 flame cells in "*Cotylophoron cotylophorum*". Porter (1938) found 19 pairs of flame cells in "*Cercaria paramphistomi calicophorum*".

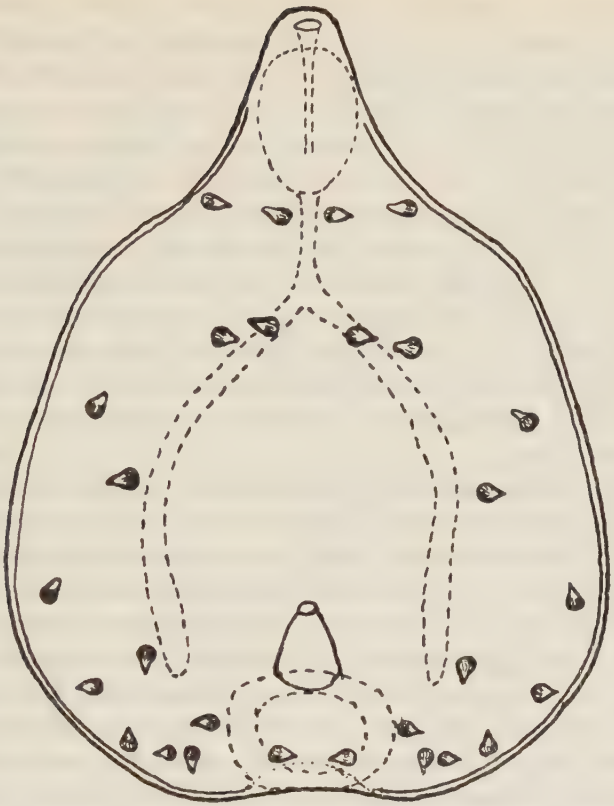


Figure 8
Cercaria: flame cell pattern.

and shifting, mostly circular, with no marked preference for the illuminated end of the container.

Identity of the cercaria

The cercaria is without dorso-lateral pharyngeal pouches, has a large anastomosis between the two main excretory ducts and develops from rediae without lateral locomotor appendages. It, therefore, clearly belongs to the "*pigmentata*" group in Sewell's classification (1922). It resembles *Cercaria Indica* XXVI fairly closely, but differs from it by being somewhat shorter, with a larger tail, by having shorter intestinal caeca and a mouth bearing papillae.

Emergence of cercariae

Several experiments were performed and numerous observations were made in

order to determine the importance and effect of such factors as light, temperatur time of day and presence of vegetation on the emergence of cercariae from the snai

Infested snails were placed singly in 1 x 4 inch glass vials, half filled with wate and properly labelled. Series of these snail-containing vials were subjected eith to sunlight, strong artificial light, subdued artificial light or total darkness for period of one hour. By the end of that time, the snails were transferred to fresh via and the process repeated over a period of 24 hours. A small quantity of 10% formal was added to each used vial, whereby the cercariae died and settled to the botto and their number as well as the number of metacercariae on the walls of the vial w carefully noted. In this manner, a record of the number of cercariae shed by snai over a 24 hour period at varying degrees of light and darkness could be obtained.

In other experiments snails were placed in individual glass vials containing wat at 28°C and 18–20°C. Small pieces of lettuce were added to some vials; others we left free of vegetation.

Results of the various experiments showed that: A. Light is the only fact stimulating emergence of cercariae. B. Cercariae are never shed in total darknes C. The presence of vegetation has no bearing on the shedding of cercariae by snail D.The temperature range of the water used in these experiments is of secondary imp tance. However, an initially larger number of cercariae emerge in water kept at 18-20° E. The number of cercariae emerging and the rapidity of such emergence is direc proportional to the intensity of the light. Most snails exposed to direct artificial watt light bulb at a distance of 30 cm) or natural light source will shed large numbe of cercariae within an hour or two after exposure. When subjected to normal diffus natural or artificial illumination, snails shed only a small number of cercariae a these may emerge irregularly and intermittently over a period of many hou F. Under strong illumination, the largest number of cercariae emerge during the secon or third hour after exposure of the snails to the light. About half the snails sta shedding cercariae within one hour after exposure to strong light, and all of the are shedding cercariae by the end of three hours of exposure. The cercaria-sheddi period is continuous and may range from two to nine hours, after which only fe or no cercariae are released. The largest number of cercariae released by any o snail over a 24 hour period was 592 (in snails that had been exposed to 20 miracid each). G. Under normal, diffused illumination, no peaks in cercarial release cou be observed, the cercariae emerging irregularly and in small numbers over a 2 hour period, with the largest number of cercariae released by any one snail being 10 H. Snails that had released cercariae under strong illumination, require a rest peri of at least 24 hours before being capable or releasing additional cercariae in a appreciable number. I. Krull and Price (1932), Krull (1934) and Bennett (193 observed that paramphistome cercariae were shed at definite periods of the da mostly between 8:30 AM and 1:00 PM. As the designated hours are also the brighte hours of the day, this periodicity of cercarial release is apparently nothing mo than a response to strong illuminattion (in this case, natural sunlight) as shown in

METACERCARIA

Shortly after release from the snail host, most cercariae commence encystation and become metacercariae. The process of encystation appears to be enhanced and accelerated by the presence of vegetation and strong light. Under strong illumination and in the presence of vegetation, up to 80% of the cercariae may encyst on the vegetation within 10 minutes after emergence from the snail. In the absence of vegetation, cercariae may encyst upon any available surface, such as the walls and bottom of a dish, pieces of cork, or strips of paper floating on the surface of the water, but encystation then is usually delayed for several hours. Encystation may also be delayed by lowering the temperature of the water containing the cercariae, or by subjecting the cercariae to subdued light or total darkness. Thus, cercariae may be prevented from encystation by placing them under 10–12°C where they remain viable for as long as 48 hours without encystation, but encyst immediately upon return to room temperature. Prolonged cooling, however, is apparently deleterious to the cercariae, as many of them are incapable of successful encystation following refrigeration.

Even under optimal conditions, not all cercariae are capable of encystation and some may undergo only incomplete encystation.

As pointed out earlier, cercariae seen to favour vegetation as encystation sites. This tendency appears to be optical rather than chemical in nature, the cercariae probably are attracted to the yellow-green colour of vegetation as suggested by Durie (1955). That this is so can be demonstrated by marking small areas on the outside of a glass container with a yellow or green wax pencil, whereupon cercariae within the container are seen to encyst mainly on the coated areas. Furthermore, areas marked with yellow grease pencil are favoured over areas similarly marked in green; lettuce leaves are favoured over deeper green-coloured vegetation. The cercariae, apparently, are responding to a narrow range of wavelengths within the spectrum of visible light.

Lettuce–leaves or other vegetation may be used as encystation surfaces for metacercariae. These, however, require constant aeration and tend to decay rapidly, and are therefore inadequate for the collection of large numbers of metacercariae. Durie (1955) described a method for collecting metacercariae on the hardened surface of a clear plastic solution (Dupont, clear methacrylate). The method used by the author was essentially that of Durie, with some minor simplifications. A 600 cc glass beaker was coated on the inside with a yellow wax-pencil and placed in a tight-fitting, thin, transparent polyethylene plastic bag. Bag and beaker were then placed in a 1000 cc. beaker, half filled with tap water containing cercaria-shedding snails. An artificial light source (60 watt light bulb) was introduced either inside or above the smaller, yellow-coated beaker. Most cercariae (over 90%) freely encysted on the plastic bag, at or near the surface of the water. It was noted, however, that the snails, when allowed free access to the plastic surface, readily fed on the encysted cercariae. A circular piece of no 18 wire mesh was therefore snugly fitted underneath the immersed beaker. The mesh prevented the snails from reaching the plastic bag, while allowing the cercariae free access to it. After an exposure period of 4–6 hours, the plastic bag, by

then coated with metacercariae, was carefully peeled off the beaker and cut into fla rectangular strips. Any number of such metacercaria-coated strips, with strips o ordinary filter paper sandwiched in between to keep them apart, could be kept im mersed in water in covered, enamel pans. To prevent bacterial growth detrimenta to the metacercariae, a small amount of antibiotic (penicillin or streptomycin was added to the water. In that manner, large numbers of metacercariae were kep viable and readily available for several months.

The method, as described, is simpler, more economical, and less time-consuming than that of Durie which requires coating the external surface of a beaker with liquid methacrylate and allowing it to dry. The introduction of the wire mesh, not used by Durie, assures a higher yield of metacercariae.

While freely swimming, cercariae show no positive phototropism, When ready to encyst, however, they accumulate mainly at points where the light is most intense Durie (1955) noted that in the absence of vegetation, most cercariae tended to encyst on the sides of a beaker near the surface of the water. In the present investigation this was found true only when the light source was at or above the surface of the water Otherwise, most cercariae encysted on the surface closest to the light source, be it the sides or bottom of the beaker.

When about to encyst, the cercaria comes to rest on its ventral surface. Strong contractions and extensions of the body follow, coupled with side movements of tail and body. These last for a couple of minutes and then cystogenous matter begins to exude from pores all over the body. This material is viscid, granular in nature and brownish in colour, containing many small, spherical granules, 1–3 μ in diameter. At this point, the tail breaks off from the body of the cercaria but remains attached to the cystogenous matter. This cystogenous matter quickly forms a brown ring around the body of the cercaria, the latter commencing to slowly turn counterclockwise

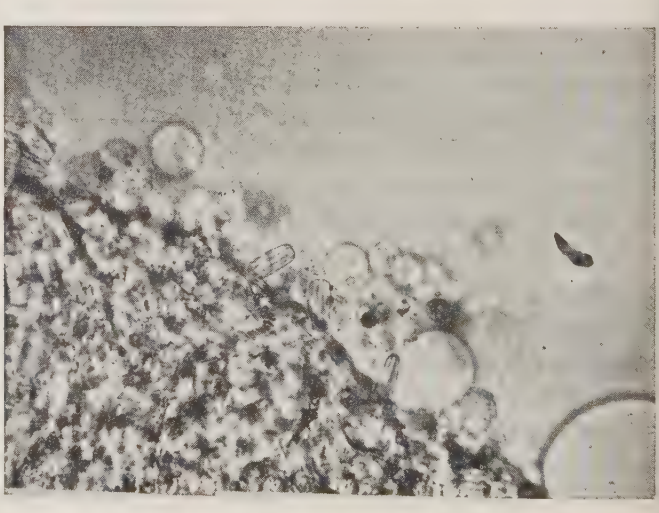


Figure 9
Microphotograph showing extrusion of cystogenous rods during process of encystation of the cercaria

within the ring. Cystogenous rods are then released through the cuticle into the surrounding granular matter (Figure 9); most rods seem to be released from the anterior half of the body. (In toto mounts of metacercariae, clear spots are scattered on the surface of the encysted larva hat suggest ducts or canals through which cystogenous rods might have been released. Furthermore, under pressure of cover slip, encysting cercariae appear to release cystogenous rods through definite channels). The movements of the cercaria within the exuded material seem to shape the latter into a dome-like cyst. Within five minutes, the exuded material forms into two, distinct layers; the outer layer, clear and unpigmented while the inner layer is opaque and brownish. The cercaria now contracts sharply and becomes spherical. The cuticle is abrup ly detached from the body, forning a third inner layer and an inner foundation for the developing cyst wall. The tail may remain attached to the cyst wall for as long as 10 hours, but usually breaks off sooner and swims away, continuing its lashing motions for several hours.

By the end of 20 minutes, a definite cyst is formed around the cercaria. The latter, however, continues rotating coun er-clockwi e (rarely clockwise) within the cyst, with occas onal side to side twists, for the next 8–12 hours. During these movements, the mouth of the cercaria is often in contact with the inner wall of the cyst (i e. cuticle) and is seen to secrete a clear fluid which may possibly function in sealing up any remaining breaks in the cuticle. The cyst wall is soft and fragile at first, but becomes hard and resilient by the end of 12 hours. By then, the encystation process is complete and all movement within the cyst apparently ceases.

In lateral view (Figure 10B) the metacercaria resembles an inverted dome-shaped cup centered on an inverted saucer, the latter structure forming the base and attachment point of the metacercaria. It measures an average 165(141–203) μ in height and 237 (222-260)μ in base diameter.

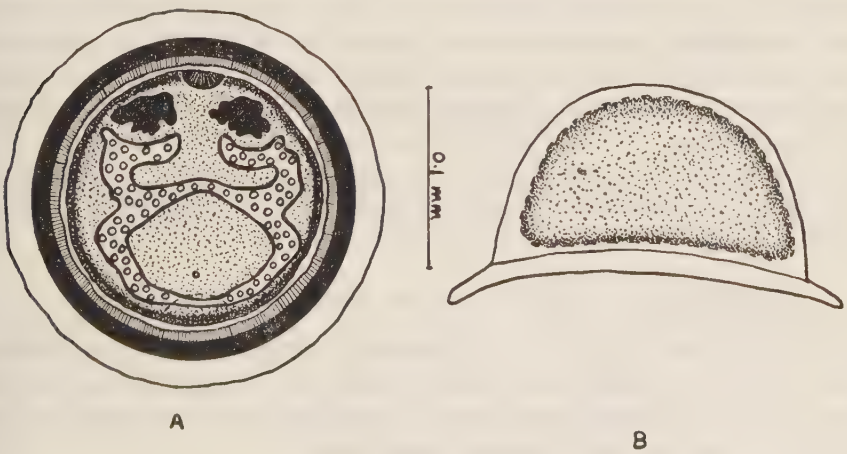


Figure 10
Metacercaria: A. en face view; B. lateral view.

Viewed en face (Figure 10A), the metacercarial cyst appears as a small sphere encircled by three concentric rings. The outermost ring, comprising the base of the cyst, is milky-white and opaque, and measures about 28 μ in thickness. The middle ring representing the cyst wall is brown, refractile, and around 9 μ thick. The third and innermost ring is the detached cuticle which encircles the embryo and is about as thick as the middle ring, differing from it only in its somewhat lighter-brown colour. Histochemical analysis showed the base of the cyst to be a muco-polysaccharide (positive reaction with toluidine blue or Lugol) while the cyst wall was essentially lipoid in nature (strong positive reaction with Sudan black). It is suggested that the base serves in anchoring the metacercaria in place while the cyst wall forms a water-tight lipoid pellicle that prevents dehydration of the metacercaria.

The roughly spherical embryo measures an average 193(178–228)μ in diameter and at first contains all the elements of the cercaria with the exception of tail and cuticle. Within a week, however, the eye-spots become indistinct, their pigment breaking up and spreading in dentritic patches. By the end of 2–3 weeks, the eyes are completely disintegrated and cannot be seen. Other structures, such as oral sucker, acetabulum, excretory ducts and intestinal caeca persist unchanged or slightly modified.

Metacercariae of a related, or possibly the same species have been kept viable by Krull (1934), for as long as five months. By placing metacercariae in a thin layer of water in a covered Syracuse dish, and changing the water daily, the present author was able to maintain some metacercariae viable for six months. All metacercariae were found viable for at least two months, following which some deaths occured. At death, the embryo was seen to shrivel up and turn gray. The cyst wall, however, persisted unchanged many months after the death of the metacercaria. By the end of four months, somewhat less than half the metacercariae survived.

It was noted earlier that newly-formed metacercariae became inactive shortly after encystation, the embryo ceasing movements and becoming immobile. However, muscular contractions and slow side to side movements of the embryo, could subsequently be induced artificially by simply placing the metacercariae under a strong light and cover slip pressure, or by gently tapping the cyst with the end of a teasing needle. These induced movements served as the criterion for the viability of the metacercaria. Viability of the metacercaria, however, was no criterion of its infectivity. Many viable metacercariae, as will be shown presently, were incapable of encystation within the final host.

EXPERIMENTAL INFESTATIONS OF SMALL LABORATORY ANIMALS

Attempts were made to infect small laboratory animals with *P. microbothrium*. Six mice and six rats were allowed to feed on small, moist pieces of bread on which hundreds of metacercariae had been pipetted. The animals were subsequently dissected at 24 hour intervals and their digestive tracts checked.

Both mice and rats were found to harbour very immature paramphistomes by the end of 24 hours.These worms were recovered from the ileum and jejunum only;

none were found in the stomach, duodenum or large intestine. The worms were small and brown-pigmented, the pigment distributed unevenly, with the dorsal surface of the worm more strongly pigmented than the ventral. The worms were capable of considerable extension and contraction. Relaxed, they were roughly pear-shaped, measuring an average 230 μ in length and 150 μ at their widest diameter. Extended, they were gourd-shaped and measured up to 500 μ in length. Morphologically, they differed little from the metacercariae; the only visible difference being a lack of any cystogenous rods in the body and the presence of doughnut-shaped ratherthan spherical excretory concrements in the excretory ducts (compare with pp. 39–40).

By the end of 48 hours only one animal out of four dissected harboured a few immature worms. These were recovered from the ileum and were identical in size and shape to the 24 hour specimens. No worms were recovered from four animals dissected 72 hours after exposure. Apparently, metacercariae of *P. microbothrium* will excyst in rodents, but the emerging worms are incapable of any development and die off or are eliminated within a couple of days.

INFECTIVITY OF METACERCARIAE

It is generally known from the literature that in experimental final host infections with metacercariae of paramphistomes the number of worms developing constitutes only a very small percentage of the number of metacercariae fed to the animal.

In an attempt to investigate this phenomenon, several series of viable metacercariae of varying ages (50 metacercariae per series) were placed in extracts of both the stomach and small intestine of mice, and incubated at 37°C. The metacercariae used were 2, 8, 11, 23 and 40 days old. A control group of 23 day metacercariae in tap water was also incubated at 37°C. The experiment commenced at 1:00 PM, and the metacercariae were checked every hour for signs of excystation. As no excystation occurred in any of the series by 9:00 PM, the metacercariae were left overnight in the 37°C incubator.

The metacercariae were checked the following morning and the subsequent observations were made:

A. No excystation of metacercariae occurred in extract of stomach.

B. Excystation of some metacercariae occurred in extract of small intestine as follows:

40 day	metacercariae	27 (54%)	metacercariae excysted*
23 day	"	29 (58%)	" "
11 day	"	22 (44%)	" "
8 day	"	4 (8%)	" "
2 day	"	no excystation	

* The criterion of excystation was the rupture of the cyst wall. It was noted that immature worms escaped through an operculum-like break in the dome-shaped surface of the cyst. None of the escaped worms were alive during the morning inspection.

C. No excystation of metacercariae occurred in the control group placed in tap water. These results suggest: 1. Excystation of *P. microbothrium* metacercariae is initiated in the small intestine of the final host. 2. At best, only about half the viable metacercariae are capable of excystation (during 24 hours). 3. Metacercariae require a certain period of development or maturation to become capable of excystation; this period is probably 10–20 days.

EXPERIMENTAL INFESTATION OF SHEEP.

Two lambs raised in a barn in a paramphistome-free locality were used for experimental infestation with *P. microbothrium*. These lambs were about three months of age when obtained. They were subsequently kept in a shed and given regular dry feed and water without being taken out to pasture. Fecal samples from each lamb were checked at regular intervals over a period of one month for presence of paramphistome eggs but none were found.

One lamb was fed a single dose of 1500 metacercariae by mouth (on July 17) and sacrificed 48 days later (September 3). A total of 60 young paramphistomids (4% of the total number of metacercariae fed to the animal) were recovered on post mortem: 38 of these from the rumen, 21 from the reticulum, and one specimen from the omasum (psalterium). None were found in the abomasum or duodenum. The young worms in all three stomachs were approximately of equal size, averaging 2.3 by 1.1 mm. They were sexually immature, with rudimentary testes, ovary and Mehlis' gland, but in other respects, quite similar to the adult worms. The muscle units of the pharynx, acetabulum, and genital atrium, the basis for Näsmark's classification system, were readily identifiable.

The second lamb was fed 500 metacercariae on the same date as the first (July 17). It started passing paramphistome eggs in its feces on October 14, i.e., 89 days after infection with metacercariae, indicating that in lambs *P. microbothrium* reaches sexual maturity within three months. The lamb was sacrificed 16 months later (Nov. 11). On post mortem a total of 225 sexually mature worms (45% of the total number of metacercariae fed the animal) were recovered from the rumen and reticulum. There were 219 worms in the rumen, mainly in its posterior half. Only six specimens were recovered from the reticulum. These were situated at the point where the reticulum opens into the rumen.

The mature paramphistome was found strongly attached to the mucosa of the stomach by means of its acetabulum, with the anterior extremity bearing the oral sucker folded backwards and underneath the body. Thus attached and densely packed one against the other, the worms resembled small red, fleshy papillae and measured 4 mm in length and 2.5–3 mm in diameter. Their extremely elastic bodies when fully extended, however, reached up to 11 mm in length, the anterior tips of the body tapering to 1 mm in diameter.

The age and size at which the worms migrated to the rumen were not determined. Bennett (1936) concludes that metacercariae of a species which he determined as

Cotylophoron cotylophorum excyst in the duodenum where they develop for 3 to 5 weeks and then migrate to the rumen.

The low recovery rate (4%) of worms, in comparison with the number of metacercariae fed to the first lamb in the present study, is possibly due to the fact that the animal was inadvertently fed fresh metacercariae. Dinnik & Dinnik (1954) obtained 14.6%, 4 6%, 15% and 15.7% recovery rates of *P. microbothrium* from four experimentally infected goats, but at the same time, obtained over 50% recovery rates from infected steers. Bennett (1936) reported low recovery rates of "*C. cotylophorum*" from experimentally infected calves (22%, 4%, 4.5% recovery rates from calves killed 21, 46 and 51 days after experimental infection).

An analysis of these citations suggests: a. that the size and species of the final host may have a bearing on the number of paramphistomes developing within it (derived from Dinnik & Dinnik), and b. that not all metacercariae excysting in the duodenum survive during migration to the rumen (based on Bennett's data).

THE MATURE WORM (Figure 11)

Mature worms were collected from the rumen of experimentally infected sheep and a naturally infected cow, the latter from the Hadera region, the same locality where

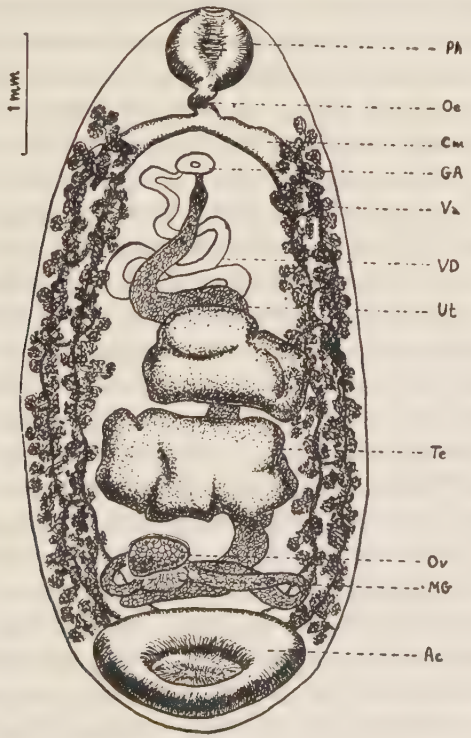


Figure 11
P.microbothrium: semi-schematic representation of sexually mature worm in ventral view

material for initial experiments was obtained. The worms were located between th villi. They were strongly attached to the mucosa by means of their powerful acetabul but were capable of considerable extensions and contractions of the body. The onse of infection in the cow could not be ascertained, but the size and sexual maturity o the recovered worms when compared with data presented by other investigator (Bennett, Dinnik and Dinnik, et al.) suggested that the worms were at least 6 month old but had not reached their maximum size as yet. Even so, specimens obtained fro the cow were on the whole appreciably larger in size than those from the lamb. Thi is in agreement with Dinnik and Dinnik (1954) who noted that *P. microbothrium* specimens recovered from experimentally infected goats were considerably smalle in size than those from experimentally infected cattle.

As mentioned previously, mature worms recovered from the rumen of experiment ally infected sheep served as a basis for identification of the species. Both staine whole mounts and stained sectioned material were used in identification and specifi diagnosis of the adult worm. Living worms were available for a short period onl and were observed unstained under low power microscope for details of the excre tory system.

While whole mounts of adult *P. microbothrium* offered a good picture of anatomic and morphological details, they were found unreliable for measuring body size an and the size of various internal organs. Measurements were therefore taken on seri sections of material fixed in Bouin, embedded in paraffin and stained with haematox lin-eosin. Material thus treated offers a constancy of size and shape not obtainab from toto mounted material. Even so, a certain amount of shrinkage does occur an should be allowed for, values obtained tending to be somewhat lower than actua ones. All measurements were based on a minimum of ten observations.

When in a dish, the adult worm is reddish in colour, conical in shape, with a grad ually tapering anterior end which terminates in an oral sucker (i.e. pharynx) an mouth, and a broad posterior end bearing a subterminal - ventral acetabulum. Bot oral sucker and acetabulum are coloured a deeper red than the rest of the body. Whe the worm is contracted, the dorsal surface is evenly curved and convex, the ventra surface slightly concave, the worm becoming roughly banana-shaped. Besides the oral sucker and acetabulum, a small genital atrium may be discernible to the naked eye, medially located on the ventral surface, about a third the body length from the anterior end. Under a dissecting microscope the excretory pore may be distinguished as a minute opening on the dorsal surface, anterior to the acetabulum. Except for these structures, no other superficial landmarks can be made out on the living worm.

As pointed out before, toto mounts were found useful in the study of gross anato mical details. The worms were flattened dorso-ventrally under pressure in 96% alcohol, stained in alum-carmine, cleared in clove oil and mounted in Canada balsam. Gross features of the digestive and reproductive systems could then be discerned. Finer details of the reproductive system, some parts of the excretory system and muscular components of the acetabulum, pharynx and genital atrium could only be

died in sectioned, stained material. The following anatomical description of *P. robothrium* is based on a composite picture formed after study of both sectioned l toto-mounted material.*

he adult worm is 3.7–6.0(5.4–6.9)mm long, 2.5–2.9(2.8–2.9)mm at its widest meter (at the level of the acetabulum) and 1.6–2.2(2.1–3)mm thick (dorso-ventral). It is covered with a thick, sparsely papillomated, longitudinally-ridged cuticle ch is continuous with the lining of the mouth opening and the duct of the excretory dder.

he digestive system resembles that of the cercaria. A narrow, transversely oval uth, about 0.28(0.35)mm across is situated at the anterior extremity of the worm leads into a muscular oral sucker (pharynx). The latter is somewhat longer than e, 0.61–0.84(0.7–0.97) mm long, and opens posteriorly into a narrow but highly tic oesophagus, 0.7–0.79 mm long, with walls that gradually thicken posteriorly without an oesophageal bulb. The oesophagus frequently curves dorsally in a oth arch and bifurcates at its posterior end to form the intestinal caeca. These are tively wide, about 0.3 mm in diameter, and after curving out in a wide arc, wind r way along the sides of the body to about the anterior margin of the acetabulum dorsal to it, where they broaden (up to 0.45 mm in diameter), bend inwards and minate obliquely, usually about in line with the middle of the acetabulum. In some cimens, however, the caeca do not extend beyond the anterior margin of the abulum and may even terminate just anterior to it. The tips of the caeca are seen oint dorsally in some specimens.

wo large, transversely oval, deeply lobed testes are situated in tandem arrangement in the intra-caecal space, occupying the middle third of the body. Each testis is eloped by a thick, fibrous sheath. The posterior testis appears somewhat larger the anterior. The testes measure 1.31–1.96 (1.6–2.65) mm in both width and so-ventral direction, and 0.62–0.94(0.8–1.3)mm in length (anterior-posterior direc-). The vasa efferentia could not be traced. The winding vas deferens, however, be discerned in the intra-caecal space anterior to the testes. It expands into a tight- oiled seminal vesicle which in turn opens into the thick-walled, coiled pars muscul- The pars prostatica is straight, about 250–270μ in length, with a wide lumen that ows and opens into the genital atrium as the ejaculatory duct. The genital atrium ocated medially on the ventral surface of the body, somewhat posterior to the rcation of the intestinal caeca. It appears en face as a protruding, concave, mus- r disc, 350 to 600μ in diameter, with a crater-like opening at its sunk center which sures 109 to 156μ in diameter and represents the genital pore.

n oval to spherical ovary is located laterally in the space between the posterior s and acetabulum and dorsal to the latter. It ranges between 515 to 770μ in

---

ize measurements on bovine material, wherever significantly different from those taken on ovine rial, are given in parentheses.

diameter. Close to it lies Mehlis' gland; it is roughly spherical in shape, measur 380–570μ in diameter, and is located dorsal and partly posterior to the ovary. T oviduct originates at the side of the ovary, continues posteriorly, and is met Laurer's canal shortly before entering Mehlis' gland. Laurer's canal is a fairly strai and narrow duct that crosses the excretory bladder before opening on the dorsal s face, somewhat off the median line and about 1.4(2.6) mm from the posterior end body. The uterus, after forming several transverse coils in the space between posterior testis and acetabulum, continues anteriorly, dorsal to the testes, then c ventrally to open at the genital atrium.The uterus is packed with eggs inthe adult wo

The vitellaria are follicular and are arranged in two wide rows, one on each side the body, close to and along the intestinal caeca. They extend anteriorly to about level of the bifurcation of the gut, and posteriorly somewhat behind the ante margin of the acetabulum. Two ducts, one from each lateral vitelline row, m dorsal to Mehlis' gland and empty into the latter through a common duct.

The excretory system is similar to that of the cercaria. An irregularly-sha saccular excretory bladder is medially located, just anterior and dorsal to the ac bulum. It lies fairly parallel to the dorsal curve of the body, measuring about (0.94) mm in length and 0.3 (0.36) mm at its widest diameter, and tapers ante into a narrow, cuticle-lined duct. The latter is about as long as the bladder and obliquely anterior and dorsal to open medially at the dorsal surface of the b usually on level with the posterior testis, 1.45 – 1.87 (2.8 – 3.27) mm from the p erior end of the body, and always anterior to the opening of Laurer's cana sagittal sections, Laurer's canal always appears to cross the excretory bladder or its and open anywhere from 0.14 to 0.38 (0.14 – 0.65) mm posterior to the excretory p

As in the cercaria, the two lateral excretory ducts open at the sides of the ex tory bladder after forming the inverted heart pattern at the center of the b (see Cercaria). They are devoid of refractive excretory concrements, however, have numerous side-branches originating along their entire length. Flame cell ciliary patches were not discerned.

The acetabulum is a strongly muscular disc, 1.4 – 1.6 (1.6 – 1.9) mm in diam and is located subterminally at the posterior extremity of the trematode. In mounts, the acetabulum appears transversly ellipsoid, i.e., somewhat wider than l

Specific diagnosis was based on Näsmark's classification system (1937), w utilizes histological details of the acetabulum, pharynx and genital atrium in dian sagittal sections of the trematode (Näsmark's nomenclature was adopte the following description and the reader is referred to the original work for the ex nation of any of the terms).*

* The present author has used Näsmark's classification system, by far the best available at pre with some misgivings, being aware that a classification based on Näsmark's histological da arbitrary and mechanical, and not necessarily of biological significance. It is the author's convi and hope that thorough and extensive investigation of Paramphistomidae may yet reveal a sou scientific basis for classification of this monotonous trematode family.

Figure 12
P. microbothrium; histological details of diagnostic importance. Median sagittal section; A. pharynx; B. genital atrium; C. acetabulum

The acetabulum in median sagittal sections is of "*Paramphistomum* type" (Figure 12C), the dorsal exterior circular muscle units (*D.E.*) showing a clear division into dorsal exterior circular 1 (*D.E.*1) and dorsal exterior circular 2 (*D.E.*2), with a sharp distinction between the units of both in size and shape. The units of the dorsal interior circular layer (*D.I.*) diminish in size toward the inside. An oblique muscle strand *D. I.* from *D. E.*; the radial musculature is diffuse, its fibers are coarse and thick, branching internally into 2–3 fibers, but without crossing over.

A study of median sagittal sections of 20 specimens shows the following distribution of circular muscle units in the acetabulum:

Dorsal exterior circular 1 (*D.E.*1)	21 (17–25) regularly spaced units
Dorsal exterior circular 2 (*D.E.*2)	6 (3– 9) irregularly spaced units
Dorsal interior circular (*D.I.*)	39 (36–44) regularly spaced units
Ventral interior circular (*V.I.*)	39 (36–43) regularly spaced units
Ventral exterior circular (*V.E.*)	17 (15–19) regularly spaced units

The pharynx (oral sucker) in median sagittal sections is of "*Paramphistomum* type" (Figure 12A) characterized by the complete absence of the middle circular layer. The pharynx is without a *pharyngeal bulb* or pharyngeal sacs. The lip sphincter and posterior pharyngeal sphincter are absent. The units of the interior circular muscle layer are small but relatively well developed. The radial muscle layer occupies a broad area.

The genital atrium is of "*Microbothrium* type" (Figure 12B). It lacks a genital sucker but has a genital sphincter and a sphincter papillae (PS). The latter is well developed and stains a deep blue with haematoxylin. The genital papilla (GP) was partially retracted in all specimens examined. The genital sphincter (GS) is inconsiderable in size but well delimited from surrounding musculature. Radial musculature (RL) is well developed, the individual fibres clearly distinguished and eventually curved. There is a suggestion of a reduced or poorly developed ventral atrium.

In morphology of the adult form, the present trematode is practically identical to *P. microbothrium* Fischoeder, 1901 (as re-defined by Näsmark, 1937), and differs from it only in the number of circular muscle units in the acetabulum, especially in *D.E.*2 series, where there are 3–9 irregularly spaced units rather than 25 regularly spaced units. It was noted, however, that only in properly sectioned material where exact median sagittal sections could be obtained, did the *D.E.*2 circular show 6– 9 irregularly spaced units. In sections not exactly sagittal or in sagittal sections on both sides of the median line, wide variations in the number of units of the *D.E.*2 circular occurred, the number of units ranging all the way from 3 irregularly spaced to 30 regularly spaced units. Such extreme variation in unit number was not encountered among any of the other acetabular unit-series, the number of units remaining constant within fairly narrow limits even in improperly sectioned material.

A DISCUSSION ON THE VALIDITY OF *P. microbothrioides* PRICE AND MCINTOSH, 1944

The basis for the creation of *P. microbothrioides* as a new species is given by E. W. Price and A. McIntosh in a short abstract published in 1944. They write: "*P. microbothrioides* is closely related to *P. microbothrium* Fischoeder, but differs from it in several respects, particularly in the number and distribution of the dorsal external acetabular muscle fibers. The dorsal-external-2 fibers (Nasmark's nomenclature) consist of about 6 to 9 irregularly spaced units in *P. microbothrioides* in contrast to about 25 regularly spaced units in *P. microbothrium*".

It is evident from the above that though several points of difference between *P. microbothrioides* and *P. microbothrium* are suggested, only one is actually described –the difference in the number and distribution of the *D.E.*2 muscle fibers in the acetabulum. We must therefore conclude that Price and McIntosh assumed this difference in number and distribution of the *D.E.*2 fibers sufficient to warrant the setting of *P. microbothrioides* apart from *P. microbothrium.* Is such an assumption justified?

In answering this question, reference is made to the following passages from Näsmark's classic work (1937). He writes (p. 308): "The description of the acetabulum being chiefly intended, in what follows, to aid the determination of sagittally sectioned Paramphistomids, it may seem somewhat precarious to base the descriptions mainly on the shape-details of the various kinds of muscles and of the muscle layers, even if these be illustrated by drawings. It is not impossible that identification by the aid of a description based on these principles would prove uncertain, and in certain cases impossible." Again, below a table showing the number of units in the various circular unit-series of 17 specimens of *P. microbothrium*, Näsmark writes (p. 309): "It is seen from the above table of *P. microbothrium* that in different specimens there seem to exist a fairly reliable constancy in the number of the units in corresponding unit-series. It is only in respect to the series of *d.e.*2 circular that three investigated specimens display strikingly deviating values." Continuing (p. 310): "But a study of the table shows that absolute constancy does not exist in any unit-series, which, of course, was neither likely nor conceivable. Thus it must not be thought that the unit figures for a particular specimen must agree with those given in the type description as a condition for identity. An approximate agreement, without striking deviation in any series figure (excepting possibly in one or two instances in *d.e.*2 circular), is sufficient to assist in demonstrating a possible identity." Finally (same page): "What mainly determines the type of acetabulum is its anatomical structure as compared with that of other types. The number of units is a secondary matter for the type-description. When the type-description mentions definite numbers for the units within the various circular unit-series, this should be taken as an example and should not be taken to mean that the figures are constant for all species of the type."

From the above, it should be clear that Näsmark used morphological characteristics of the acetabulum to identify acetabular types, not species of Paramphistoma.

Full identification of a particular species was based on characteristics of acetabulum pharynx and genital atrium and, where necessary, further anatomical details. Wha Näsmark calls the "Paramphistomum" type of acetabulum is typical for all speci of genus *Paramphistomum* and cannot serve as a criterion for separating th various species within the genus.

In the present study, as has been noted earlier, the constancy of the small number o the *D.E.*2 circular units in the acetabulum is manifest only on exact median sagitta sections. Otherwise, the number of units may range to 30, that is to say, approac or approximate the value given by Näsmark for *P. microbothrium*. Furthermore an examination of specimens from Dinnik and Dinnik's collection of *P.microbothriu* and Bennett's collection of "*Cotylophoron cotylophorum*" (*i.e. P. microbothrioides*) revealed variability in the number of D.E.2 circular units similar to that encountere in the present material. Thus, in Dinnik and Dinnik's material (20 specimens examined the number of the D.E.2 circular units varied between 1 and 34, with a range of 1–1 at the median sagittal point, while Bennett's material (only 8 specimens available the present author) varied between 2 and 17, with a range of 2–6 at the exact media sagittal point.**

Material in all three groups also showed consistency in the following two respec

1. As the number of the D.E.2 circular units increased, their size diminished a they tended to become more regularly distributed.

2. An increase in the number of units of the D.E.2 circular went hand in ha with a corresponding decrease in the number of the D.E.1 circular units and vi versa.

These facts coupled with the difficulty of obtaining exact median sagittal section cast, in the author's opinion, considerable doubt on the diagnostic significance o the number of units in the D.E.2 circular layer.

If we are to adhere strictly to Näsmark's views and on the basis of the abov findings, we must conclude that there are no valid grounds for the creation of *P. microbothrioides* as a new species, distinct from *P. microbothrium*. The two should rather be considered synonymous, the latter name having priority. The Israel species described herewith is therefore regarded as *Paramphistomum microbothrium* Fisch oeder, 1901.

Price and McIntosh (1944) consider the species described as *Cotylophoron cotylophorum* by Bennett (1936) to be identical with *P. microbothrioides*. A comparison of

* The author wishes to express his gratitude to Dr. J. A. Dinnik, of the East African Veterinary Research Organization, for kindly providing material from his African collections, and to Dr.Turner, of the University of Illinois, Urbana, Illinois, for sending specimens collected by Dr. Bennett in the U.S.A.

** A. Kotlan (1958) offers a brief description of *P. microbothrioides* from cattle in Hungary, based on examination of 5 specimens only. While there is no need at this point to enter into a debate as to the value of a description based on such a small number of trematodes, it is of interest to note that the number of D. E. 2 circular units in the 5 specimens described varied between 6 and 12.

e species under present investigation with that of Bennett's shows an overall agreement between the two descriptions in regard to anatomical and morphological details of the adult and larval stages of the trematodes. In the present investigation, however, the eggs and all larval stages except the redia, are consistently larger in ze. Possibly, these size differences might be due to varying ecological conditions, might indicate variation within the range of the species. In respect to size, the gs and larval stages are in perfect agreement with those reported by Dinnik and innik (1954) for *P. microbothrium* in Kenya.

Bennett reports only a single case of daughter-redia production in his material, hile in the present material and that of Dinnik and Dinnik, daughter rediae form n essential phase in the life-cycle of the trematode. Durie (1953) suggests the possibiy that a sudden drop in temperature may stimulate the production of daughter diae in infected snails. As sudden drops in the temperature of aquaria containing e intermediate host snails are not reported by Dinnik and Dinnik and have not ccurred in the experiments of the present author, this possibility may be ruled out. is possible, however, that the type and nature of the intermediate snail host (*Fossaria* ails in Bennett's investigation as opposed to *Bulinus* snails in Dinnik and Dinnik nd the present studies) may be the determining factor in production of daughter diae, and might even have a bearing on the size of the larval stages developing ithin it.

KEY TO THE GENUS *PARAMPHISTOMUM*

The following key was composed to provide a simple, rapid means of identifying the arious species of genus *Paramphistomum* Fischoeder, 1901, in accordance with äsmark's classification. Näsmark (1937) originally included 11 species in the genus. n bringing the present key up to date, several new species have been added. Only pecies whose diagnosis is based on Näsmark's classification system, were included. hus, *P. maplestoni*, *P. cuonum*, *P. magnum*, *P. spinicephalus* and other suggested new pecies of questionable validity, are disregarded. It should be mentioned that Skrjabin's 1949) comprehensive work on paramphistomes was found of great benefit in comosing the present key. The reader is once more referred to Näsmark (1937) for xplanation of nomenclature.

(2) Testes distinctly diagonal.. .. .. *P. skrjabini* Popowa. 1937*
(1) Testes tandem or almost tandem.. 3
(4) Dorsal exterior circular muscle units in the acetabulum *not* differentiated into two distinct groups.. .. .. *P. sukari* Dinnik, 1954.
(3) Dorsal exterior circular muscle units in the acetabulum differentiated into two distinct groups.. 5
(18) Pharynx of "Paramphistomum" type (No pharyngeal bulb; no primary pharyngeal sacs; middle circular muscle layer absent).. 6

* I. V. Davidova (1958) questions the validity of *P.skrjabini* and considers it identical with *Calicophoron calicophorum.*

6 (9) Sphincter papillae in the genital atrium absent.. .. ..
7 (8) Radial musculature in the genital atrium absent.. .. *P. gracile* Fischoeder, 190
8 (7) Radial musculature in the genital atrium present .. .. *P. epiclitum* Gischoder, 190
9 (6) Sphincter papillae in the genital atrium present.. .. ..
10(11,12,17) Genital sphincter present.. .. ..
11(10,12,17) Genital sphincter very strong.. .. .. *P. clavula* Näsmark, 193
12(10,11,17) Genital sphincter small and inconspicuous.. .. ..
13 (16) Ventral atrium small (poorly developed).. 14
14 (15) Dorsal exterior circular 2 muscle units in the acetabulum are 25 in number and regular spaced.. .. .. *P. microbothrium* Fischoeder, 190
15 (13) Ventral atrium very large .. .. *P. bothriophoron* (M. Braun, 1892) Fischoeder, 190
16(10,11,12) Genital sphincter absent .. .. *P. ichikawai* Fukui, 192
17 (5) Pharynx of "Liorchis" type (No pharyngeal bulb; no primary pharyngeal sacs; midd circular muscle layer present) .. ..
18 (23) Sphincter papillae in the genital atrium absent.. .. ..
19 (20) Radial musculature in the genital atrium present.. .. .. *P. leydeni* Näsmark, 1937; *P. scotiae* Willmott, 1950.**
20 (19) Radial musculature in the genital atrium absent.. 2
21 (22) In acetabulum: 19 dorsal exterior 1 circular units; 31 dorsal interior circular uniti 49 ventral interior circular units.. .. ..*P. gotoi* Fukui, 192
22 (21) In acetabulum: 14 dorsal exterior 1 circular units; 41 dorsal interior circular uniti 40 ventral interior circular units.. .. .. *P. cervi* (Zeder 179
23 (18) Sphincter papillae in the genital atrium present.. 2
24 (25) Sphincter papillae in the genital atrium poorly developed; circular and radial muscu ture absent .. .. *P. liorchis* Fischoeder, 19
25 (24) Sphincter papillae in the genital atrium well-developed; circular and radial musculat present.. .. .. *P. hiberniae* Willmott, 19

ABBREVITATIONS USED

Ac — Acetabulum.
AG — Apical gland.
BM — Brain mass.
BP — Birth pore.
Cm — Intestinal caecum.
CP — Ciliary patch.
CT — Caudal excretory tube.
*DE*1 — Dorsal exterior 1 circular units.
*DE*2 — Dorsal exterior 2 circular units.
DI — Dorsal interior circular units.
EB — Embryo ball.
ExB — Excretory bladder.
ExD — Excretory duct.
EC — Excretory concrements.
EP — Excretory pore.
ES — Eye spot.
FC — Flame cell.

** *P. papilligerum* Stiles and Goldberger, 1910, belongs to this group, but it is not introduced int this key for its description lacks the details upon which the differentiation of species herewith is based

*** Willmott (1950:168) points out that there is a similarity between the species *leydeni* and *scotia* but that there are "a number of other morphological differences between them". We fail to recogniz these differences but would refrain from identifying these species as long as the number of muscl bundles in the acetabulum of *P. leydeni* is unknown.

GA — Genital atrium.
GE — Germinal epithelium.
GP — Genital papilla.
GR — Genital primordia.
GS — Genital sphincter.
GT — Germinal tissue.
IC — Interior circular layer.
MF — Mucoid Fin.
MG — Mehlis' gland.
MR — Muscle ring.
NC — Nerve cell.
NCN — Nerve cell nucleus.
Oe — Oesophagus.
OS — Oral sucker.
OV — Ovary.
PG — Penetration gland.
Ph — Pharynx.
PS — Papillar sphincter (i.e. sphincter papillae).
RG — Rhabdocoel gut.
RL — Radial muscle layer.
SB — Sensory bristle.
SG — Salivary gland.
SN — Subepithelial nucleus.
SP — Sensory papilla.
Te — Testis.
Ut. — Uterus.
Va — Vitellaria.
VD — Vas deferens.
VE — Ventral exterior circular units.
VI — Ventral interior circular units.

REFERENCES

1. Barlow, C. H., 1925, The life cycle of the human intestinal fluke *Fasciolopsis buski* (Lankester), *Amer. Jour. Hyg.* Monographic Series, No. 4.
2. Baugh, S. C., 1954, Studies on larval flukes of *Vivipara bengalensis* (Lamarck). Part I. On a new furcocercous cercaria of the *Vivax* type, *Jour. Zool. Soc. India*, **6**,2, 123–128.
3. Bennett, H. J., 1936, The life history of *Cotylophoron cotylophorum*, a trematode from ruminants, *Illinois Biol. Monog.*, **14**,4, 1–119.
4. Davidova, I. V., 1958, On the identification of species causing paramphistomiasis in animals in U.S.S.R., *Trudi Dokl. Meet. All-Soviet Assn. of Helminthol.*, Moscow, Dec. 1958.
5. Dawes, B., 1960, The penetration of *Faciòla hepatica* into *Limnea truncatula* and of *F. gigantica* into *L.auricularia*, *Trans. Roy. Soc. Trop. Med. and Hyg.*, **54**, 1, 9–10.
6. Dinnik, J. A. and Dinnik, N. N., 1954, The life-cycle of *Paramphistomum microbothrium* Fischoeder, 1901 (Trematoda, Paramphistomidae), *Parasitology*, **44**, 3&4, 285–299.
7. Durie, P. H., 1953, The paramphistomes (Trematoda) of Australian ruminants. II. The life history of *Ceylonocotyle streptocoelium* (Fischoeder) Nasmark and of *Paramphistomum ichikawai* Fukui, *Australian Jour. Zool.*, **1**,2,193–222.
8. Durie, P. H., 1955, A technique for the collection of large numbers of *Paramphistomum* (Trematoda) metacercariae, *Australian Jour. Agric. Res.*, **6**, 200–202.
9. Fain, A., 1952, Description de la cercaire de *Schistosoma intercalatum* Fischer, 1934 et d'une nouvelle xiphidiocercaire du groupe Ornatae (sous-groupe Prima), *Ann. Soc. Belge de Med. Trop.*, **32**,5, 433–444.
10. Fain, A.; Thienpont, D.; Herin, V. et Deramee, O., 1953, Observations sur *Schistosoma rodhaini* Brumpt au Ruanda — Urundi et description de la cercaire de ce schistosome, *Ann. Soc. Belge. de Med. Trop.*, **33**, 5, 423–444.

11. Fishoeder, F., 1901, Die Paramphistomidender Saeugetiere, *Zool. Anz.*, **24**, 365–325.
12. Fishoeder, F., 1904, Beschreibung drerier Paramphistomidenarten aüs Saeugetieren, *Zool. Jahrb. Syst.*, **20**, 453–420.
13. Gordon, R. M.; Davey, T. H. and Peaston, H., 1934, The transmission of human Bilharziasis in Sierra Leone, with an account of the life-cycle of the Schistosomes concerned, *S. mansoni* and *S.haematobium.*, *Ann. Trop. Med. and Parasit.*, **28**, 323–418.
14. Kotlan, A., 1958, Zur Kentis der Paramphistomiden Ungars. *Acta Vet. Hung.*, **8**, 93–104.
15. Kruidenier, F. J., 1953a, Studies on mucoid secretion and function in the cercaria of *Paragonimus kellicotti* Ward (Trematoda: Troglotrematidae), *Jour. Morph.*, **92**, 3, 531–544.
16. Kruidenier, F. J., 1953b, Studies on the formation and function of mucoid glands in cercariae: Opisthorchoid cercariae, *Jour. Parasitol.*, **39**, 4, 385–391.
17. Krull, W. H., 1932, Studies on the life history of *Cotylophoron cotylophorum* (Fischoeder, 1901) Stiles and Goldberger, 1910, *Jour. Parasitol.*, **19**, 165–166.
18. Krull, W. H., 1934, Life history studies on *Cotylophoron cotylophorum* (Fischoeder, 1901) Stiles and Goldberger, 1910, *Jour. Parasitol.*, **20**, 173–180.
19. Krull, W. H. and Price, H. F., 1932, Studies on the life history of *Diplodiscus temperatus* Stafford from the frog, *Occ. Pap. Mus. Zool. Univ. Mich.*, No. 237.
20. Näsmark, K. E., 1937, A Revision of the trematode family *Paramphistomidae*, *Zool. Bidr.* Uppsala, **16**, 301–565.
21. Nobel, T. A., 1956, Histopathology of paramphistomiasis, *Refuah Veterinarith*, Jerusalem, **13**, 4, 204–206.
22. Porter, A., 1938, The larval trematoda found in certain South African Mollusca, *Pub. South Afric. Instit. Med. Res.*, **42**, 8, 1–492.
23. Price, E. W. and Mcintosh, A., 1944, Paramphistomes of North American domestic ruminants (Abstract), *Jour. Parasitol.*, **30**, 9, August Suppl.
24. Rowan, W. B., 1956, The mode of hatching the egg of *Fasciola hepatica*, *Exp. Parasitol.*, **5**, 118–137.
25. Sewell, R. B. S., 1922, Cercariae Indicae, *Ind. Jour. Med. Res.*, **10**, Suppl.
26. Skrjabin, K. I., 1949, Podotryad Paramphistomata (Szidat, 1936) Skrjabin and Schulz, 193 (Russian text) In: *Trematody Zhivotnykh i Chelovieka*, **3**, Moscow, Ac. Sci. U.S.S.R.
27. Tandon, R. S., 1957, Life History of *Gastrothylax crumenifer* (Creplin, 1847), *Zeitschr. wiss Zool.*, **160**, ½, 39–71.
28. Ullman, H., 1954, Observations on a new cercaria developing in *Melanopsis praemorsa* in Israel, *Parasitology*, **44**, 1&2, 1–15.
29. Vercammen-Grandjean, P. M., 1951, Sur la chaetotaxie de la larve infestante de *Schistosoma mansoni*, *Ann. Parasitol. Hum. et Comp.*, **26**, 412–414.
30. Willmott, S., 1950, On the species of *Paramphistomum* Fischoeder, 1901, occurring in Britain and Ireland with notes on some material from the Netherlands and France, *Jour. Helminth.*, **24**, 155–170.

THE BIOLOGY OF *CALLIPTAMUS PALAESTINENSIS* BDHMR. WITH SPECIAL REFERENCE TO THE DEVELOPMENT OF ITS EGGS

M. P. Pener and A. Shulov
Department of Zoology, The Hebrew University of Jerusalem

ABSTRACT

The embryonic development of *Calliptamus palaestinensis* Bdhmr. was studied at 20°C, 27°C and at room temperature. The eggs, laid into dry soil, were moistened in 7 groups after various periods of time since oviposition (0, 30, 85, 120, 150, 170, 200 days). One more group of eggs was kept continuously in dry soil. The embryonic stages were checked at intervals. It was found that the embryos pass through three distinct stages designated as predormant, dormant and postdormant periods. The first and third periods can be influenced by temperature, whereas the middle one depends on the addition of water and the influence of the low temperature without which further development is not possible.

The embryonic development of *C. palaestinensis* fits the ecological conditions of Israel well. The eggs were laid in dry soil and either remained dry for some months or were moistened by rain. In both cases they developed slowly and, after a certain dormant period, resumed their development, bringing the hoppers into the most favourable conditions of food and climate in the middle of spring.

INTRODUCTION

There are some 15 species of *Calliptamus* distributed in North Africa, the South of Europe, the South-European islands, Madera, the Canary Islands and Asia excluding its Northern and South-Eastern parts) (Bei-Bijenko and Mishchenko 1951). Ramme (1951) considers only 12 species.

According to Bodenheimer (1937) there are 3 species in Israel including *C. palaestinensis* Bdhr., *C. siculus* Burm (= *barbarus* Costa), *C. siculus deserticola* Voss. (= *barbarus deserticola* Voss) and *C. italicus* Br. (= *italicus* L.). We have been unable to find any *C. italicus* in all the collections of this genus from Israel and believe it to be a misidentification. However, *C. palaestinensis* and *C. barbarus* (this latter with two subspecies) are present here. It is also possible that there is one more form of *Calliptamus* which seems to be related to *C. barbarus*. This form is found in the Negev and will be described elsewhere.*

C. palaestinensis is found in the Mediterranean region of Israel. Bodenheimer (1935) mentioned the time of appearance of various stages of *C. palaestinensis*. According to him, the hoppers appear in the late spring (April, May) and the adults

* Specimens of these Israeli forms of *Calliptamus* are now being investigated at the Anti-locust Research Centre.

Received April 26, 1960

are to be found from June, their number being gradually reduced from Octobe till January.

Uvarov (1928) mentions the gregarious habits of the females of *C. italicu* during the oviposition. According to the best of our knowledge, no exact stud regarding the conditions and ways of egg development in this genus has bee published (Andrewartha, 1945).

MATERIALS AND METHODS

We had at our disposal material included in the collections of the Department o Zoology, Hebrew University since 1927 and those collected by us during 1952 1958. For the purpose of comparison the collections of Mr. Fishelsohn (Universit of Tel Aviv) and that of Dr. J. Wahrman have been consulted.

In order to have egg-pods in sufficient numbers, an extensive collection o *C. palaestinensis* has been carried out twice (1954 and 1955) from a small area i the vicinity of Jerusalem.

The grasshoppers were kept in wooden cages slightly different from those in us at the Anti-Locust Research Centre, London (Hunter-Jones, 1956). Electric bul were provided for light and warmth and kept lit permanently. The food consist of fresh grass every day and an ample supply of "Quaker" oats in petri-dish

The tin vessels for egg-laying were filled with a dry mixture of sand and hea soil sieved through a mosquito wire-netting and checked every day in the lat hours of the morning. The egg-pods found were considered as 0 days old.

The experiments on egg development were carried out in three series: A. a room temperature, B. at 20°C (± 1°C) and C. at 27°C (± 1°C). The two consta temperatures, as above, were maintained by thermostats.

The egg pods were placed into glass test tubes (9.5 × 2.2 cm) filled with th above mentioned mixture of sand and soil and closed with cotton plugs. On group of egg pods was kept permanently in dry soil at each temperature and othe groups were moistened at fixed periods at the same temperatures and kept in th wet soil until the end of the experiment.

In each group, samples of eggs (40 each time) were taken out of two to five eg pods at fixed intervals until hatching. At the end of each experiment (when considerable time elapsed since the last hatching) the remaining eggs were checke and the percentage of hatchability calculated.

The eggs extracted from the egg pods as above were killed in hot Bouin fluid and their embryological stages identified according to the scheme devised by Bodenheimer and Shulov (1951) for *Dociostaurus maroccanus*. This scheme was slightly changed in a way that stage XX of the above scheme was split into three stages as follows:

XX. The embryo fills the whole egg and its head reaches the micropyle.

XXI. The hind legs become elongated. Conspicuous rows of spines appear on the dorsal surface of the hind tibia.

XXII. The embryo is nearly ready for hatching, its integument is almost fully pigmented especially in legs and mandibular teeth. At the distal lateral sides of the hind femora, crescent-like heavy pigmented areas make their appearance. The tibial spines become dark in colour.

RESULTS

. *Observations on the Life Cycle*

The adults of *C. palaestinensis* were found in the Galilee, on Mt. Carmel, on the western slopes of the Samarian and Judean hills, in the valley of Ezdrelon and in the coastal plain. The first adults in the field were found from the end of May (Costal Plain) to June (Jerusalem) and the last ones in the beginning of March (Har-Tuv). The maximal number of adults was found from June until November. The number of individualis in the field markedly decreases from December.

The young hoppers were found on many occasions, but only those from Jerusalem were identified with accuracy as they were kept until the last moult. They were found in the field from 9.V until 16.VI. As these hoppers belonged to their last and last but one nymphal instars, it appears that the hatching occurred mainly in April.

Considerable quantities of adult grasshoppers were collected in the field around Jerusalem throughout the period of July to November (mainly from August to October) and kept in the cages for oviposition. During 1955, 319 egg pods were laid from IX till 21.XII. In a previous experiment carried out in 1953 a few egg pods were laid even in the second half of August.

Some of the hoppers which hatched in the laboratory during IV..55 were bred individually until the last moult and the time of their development recorded. The temperature in the cages was maintained at approximately 30°C, however the fluctuations were $\pm$ 5°C due to changes of the temperature of the room. The average rate of development from hatching until imago for 8 males is 39 ± 3.7 days (range 32—43). The same figures for 7 females are 50 ± 8.0 days (range 47—61 days).

The statistical analysis (two sided t-test) showed that the rate of development of the males was significantly different from that of the females ($t_{14} = 3.84$, $P < 0.002$) i.e. the development of females is slower.

Most of the adult *Calliptamus* died in the laboratory before the end of the calendar year and only few individuals remained alive during the first half of January.

The last survivors in the laboratory as well as those found in the field were females.

. *Description of the eggs and egg pods*

The fresh-laid eggs *C. palaestinensis* are of "normal type" (descriptions according to Chapman and Robertson, 1958), their measurements being length (in mm):

4.54 (average); max. 4.94, min. 4.07 (n: 106). Width (in mm): 1.28 (average); max 1.40, min. 1.10 (n: 106). The mean weight of the egg at oviposition is 4.50 m (n: 129), after 50—60 days in dry soil 4.26 mg (n: 171), at hatching — 10.14m (n: 19). The sculpture of the chorion is shown in Figure 1.

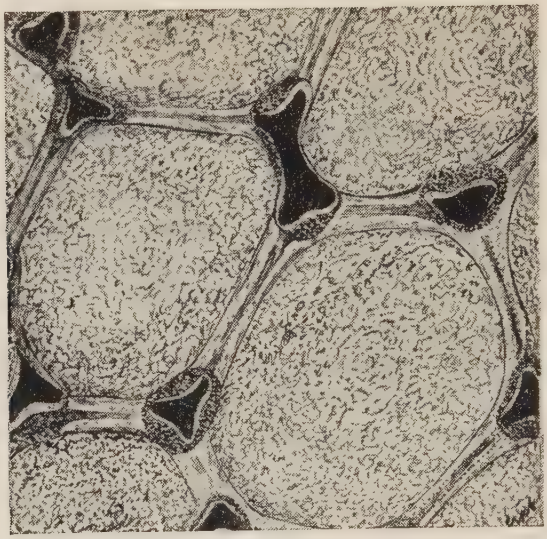


Figure 1. The sculpture of the chorion, magnification 1,000 ×.

The investigation of 281 egg pods has shown that the average number of egg in one egg pod is 30.41, the fluctuation being between 7 and 51. The distribution o the number of eggs in the egg-pod is according to the normal distribution curve (see Figure 2). Three egg-pods have been found empty.

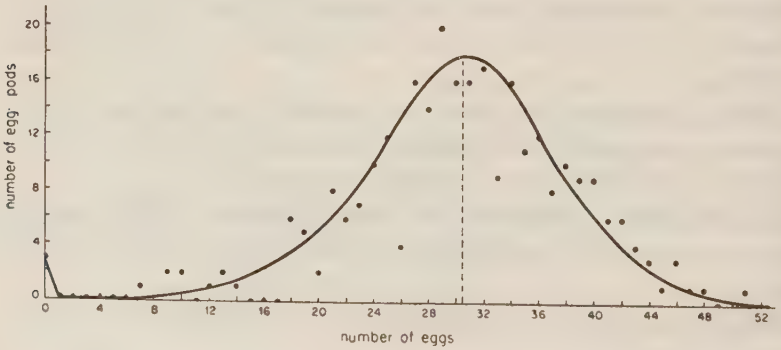


Figure 2
The distribution curve of the number of eggs in the egg pods of *Calliptamus palaestinensis*
Abscissa: number of eggs.
Ordinate: number of egg pods.

A typical egg–pod consists of two parts, a plug and an egg mass. Both parts are covered externally by soil particles. The eggs are embedded in a froth–like substance which is confined in a very hard parchment–like envelope. The soil particles are strongly glued to this envelope forming a hard core well preserved in the dry egg pods. The egg mass is cylindrical and slightly curved. The eggs are arranged in several parallel rows inclined in approximately 45° to the vertical axis of the egg pod (Figure 3). The plug is separated from the egg mass by a clear constriction.

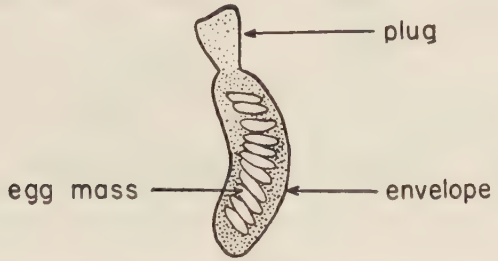


Figure 3. Diagram of the egg pod showing plug, egg mass and envelope.

In only one egg pod out of more than 300, the plug also included eggs. The form of the plug varies widely from an acuminated slender cap to a rounded, nearly globular one (see Figure 5). The plug remains soft in all types of soil. The upper end of the plug does not always reach the surface soil level.

The following table represents the measurements of several egg–pods extracted after oviposition from the dry s il. The method of measuring is shown in Figure 4.

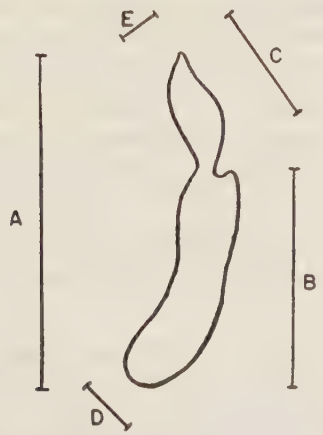


Figure 4. Method of measuring of the egg pod.
A. The length of the egg pod.
B. The length of the egg mass.
C. The length of the plug.
D. Maximum width of the egg mass with envelope.
E. Maximum width of the plug.

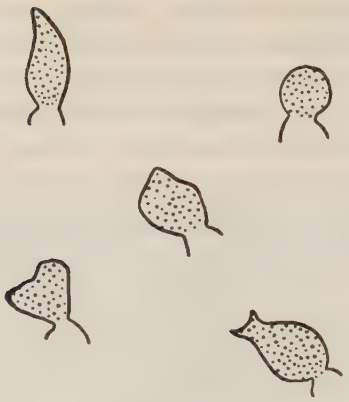


Figure 5. Various forms of the plug of the egg pod.

TABLE 1

The measurement of the O day old egg pods of Calliptamus palaestinensis Bdhmr. extracted from the dry soil.

Objects measured	Mean in mm	Maximum in mm	Minimum in mm	No. of egg pods used for measurements
The length of the egg pod	23.04	30.4	16.8	67
The length of the egg mass with the envelope	18.08	26.6	12.3	81
The length of the plug	6.30	14.3	2.5	68
Maximum width of the egg mass with envelope	5.96	7.9	4.9	81
Maximum width of the plug	4.39	5.8	2.8	67

When the egg pod is moistened, its envelope becomes soft and loose and its form becomes less definite.

3. *Studies on developments of eggs under known conditions*

Three series of experiments were carried out, at 20°C, 27°C and at room temperature (see Material and Methods). One group of egg pods in each series was kept in dry soil for the entire duration of the experiment, another kept constantly in damp soil, and still other groups were transferred from dry into wet soil at fixed intervals and kept there until the end of the experiment.

In an additional experiment, one group of egg pods has been moistened on the

31st day after oviposition and was transferred later from room temperature to 29°C.

The development of the eggs was followed by a constant sampling of eggs in each group (see Material and Methods). In all the groups of the egg pods transferred from dry to wet soil, the first sample was taken only after the moistening, under the assumption that the earlier embryonic stages could be considered as similar to those found among the eggs kept permanently in dry soil. In each group the time and number of hoppers hatched as well as the condition of those eggs which did not hatch were noted.

The results of the investigation on the development of eggs are cited in three Tables (3, 6, 8) for the three temperatures of the main experiments. These tables show the rate of development of the eggs according to the time of transfer into the damp soil and the period elapsing between the oviposition and sampling. The set of data for each combination of factors includes:

A. The average embryonic stage (calculated from all normal embryos investigated at the same set of conditions).

B. The range of embryonic stages found among the sampled eggs.

It must be explained, however, that the average stage of development of the embryo is a mathematical abstraction, as the stages of development do not last for the same periods of time. Thus, it may occur that practically no embryo is found in the stage designated as an average one for a given set of conditions.

The number of nymphae hatched at room temperature and at 20°C is shown in Tables 4 and 7 respectively. In these tables the results have been arranged according to the period elapsing between oviposition to hatching in 8 groups in which the eggs have been transferred into soil. The egg pods were checked daily and the results summed up from 10 days intervals. Some of the nymphs which hatched during the time of experiment did not creep out of the soil. Although not found during the routine daily examination, they were found and counted later, when the whole content of the glass test tubes was turned out and minutely investigated. Those nymphs are cited in a separate column in the above tables and their number taken into account in the calculation of the percentage of hatching.

The dates of hatching of *C. palaestinensis* observed at room temperature are summed up in Tables 5a and 5b, together with the dates of oviposition of the same egg pods according to the groups of various periods of moistening.

The experiments were carried out during two successive years; those of 1954/55 being considered as preliminary experiments, and those of 1955/56 as the main one. The results obtained during both years agreed well.

A. Observations of egg development at room temperature

Tes -tubes containing egg pods were kept on the open shelves of a room. Fluctuations of temperature were recorded by a maximum–minimum thermomet once a day and summarized in Table 2.

TABLE 2

Temperatures of the room in which the experiments on the egg pods of Calliptamus palaestine sis were carried out

a. 1954/55

Month	Mean temperature in °C	Highest temperature during the month in °C	Lowest temperature during the month in °C
September	25.5	29	22
October	24.0	28	20
November	20.5	25	15
December	18.1	23	13
January	18.1	22	14
February	18.0	23	13
March	17.9	24	12
April	20.5	30	13
May	22.7	30	15
June	27.0	35	21
Average for the whole season	21.2		

b. 1955/56

Month	Mean temperature in °C	Highest temperature during the month in °C	Lowest temperature during the month in °C
October	26.4	29.5	20.5
November	20.5	26.5	14.0
December	18.1	28.0	11.0
January	17.2	26.0	9.0
February	16.4	24.5	8.5
March	17.1	23.5	11.0
April	19.7	28.0	10.5
May	22.0	29.0	15.5
June	26.6	33.0	19.0
Average for the whole season	20.4		

The resul's of the obsetvations on the development of the eggs are summarized in Table 3, those on hatching in Tables 4a and 4b (for the seasons 54/55 and 55/56 respectively) and those on the dependence of the date of hatching upon the date of oviposition and upon the time of moistening in the Table 5a for 1954/55 and in Table 5b for the 1955/56 season.

Table 2 shows that the temperature fluctuated from 12°C to 35°C during the season of 1954/55 and from 8.5°C to 33°C during 1955/56. The maximal range of fluctuation for one month ocurred in April (17°C in 1954/55 and 17.5°C in 1955/56). The average temperature of the months fluctuated between 17.9°C (March) and 27°C (June) in 1954/55, and from 16.4°C (February) to 26.6°C (June) for the years 1955/56. The total average of the room temperature during the observations was 21.2°C for 1954/55 and 20.4°C for 1955/56.

Table 3 shows that eggs kept continuously in dry soil develop in 50 days up to the stages XI–XIV (late anatrepsis). After 120 days they shrink and begin to die in this stage if not moistened. However, it must be stressed that the shrunk embryos are not necessarily non-viable. Although after 120 days all the embryos kept in the dry soil were designated shrunken, some eggs resumed their development in the parallel experiments even if moistened on the 170th day after oviposition. In the year 1955/56 no egg was found able to resume its development when kept in dry soil 200 days after oviposition and later moistened. In the year 1954/55 similar results were obtained for eggs kept for more than 218 days in dry soil.

Eggs kept continuously in damp soil develop within 50 days up to stages XI–XIV. The katatrepsis began between the 70th and the 100th day, and the last eggs of the stage XIV (i. e. just before katatrepsis) were found on the 120th day. The hatching began at the 156th day after oviposition in 1955/56.

When the soil containing the egg pods is moistened 30 days after oviposition, the eggs develop similarly to those kept permanently in damp soil. When moistened on the 85th day, a slight delay if any, could be seen. When moistened on the 120th day, the influence of the delayed moistening upon the rate of development was already markedly pronounced in the delay of katatrepsis and of hatching. A further delay in the time of moistening to the 150th and the 170th day shows additional delay both at the time of katatrepsis and at that of hatching.

Tables 4a and 4b show how the duration of development and the percentage of the hoppers hatched were influenced by the time of moistening. It appears that this influence is slightly different in 1954/55 than in 1955/56. While during the first season the marked decrease in the percentage of hoppers hatched was seen in groups of egg pods moistened on the 170th day, during the second season this decrease already begins within the groups of the egg pods moistened on the 150th day. There is also some difference in the duration of development in experiments conducted during both seasons. The mean duration of development during the first season was 196–204 days for the group of egg pods kept permanently in damp

Successive stages of development of the eggs of Calliptamus palaestinensis in the season 1955/56 at room temperature. The results are arranged according to the groups of egg pods moistened at various intervals after oviposition. The figures included in each square are based upon the examination of some 40 eggs taken out of 2—5 egg pods. The upper figure (A) in each square represents the average embryonic stage, the lower one (B) — the range of embryonic stages found among the sampled eggs.

Days elapsed between oviposition and moistening / Days elapsed between oviposition and fixation of the eggs		10	15	20	30	40	50	70	100	120	140	160	180	200
0 (=Eggs kept permanently in damp soil)	A	3.2	4.9	3.4	9.5	9.5	10.7	12.7	16.8	19.6	20.3	*21.2		
	B	I–IV	I–VII	I–VI	VIII–XI	VIII–XI	VIII–XIV	XI–XIV	XIV–XX	XIV–XXI	XX–XXI	XX–XXII		
Eggs moistened on the 30th day after oviposition	A	↓	↓	↓	↓	9.5		13.1	16.2	18.2	20.8	*21.6		
	B					VIII–XI		XI–XIV	XIV–XX	XIV–XXI	XX–XXI	XXI–XXII		
Eggs moistened at the 85th dayafteroviposition	A	↓	↓	↓	↓	↓	↓	↓	13.9	18.8	20.5	*20.6	21.1	
	B								XIII–XIV	XIV–XX	XX–XXI	XIX–XXI	XX–XXII	
Eggs moistened on the 120th day after oviposition	A	↓	↓	↓	↓	↓	↓	↓	↓	↑	17.9	20.0	*20.8	
	B										XIV–XIX	XIV–XXI	XX–XXI	
Eggs moistened on the 150th day after oviposition	A	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	12.0	18.2	*
	B											XII	XII–XX	
Eggs moistened on the 170th day after oviposition	A	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	13.5	120.0*
	B												XII—XIV	XIV—XXI
Eggs not moistened at all (kept in dry soil since oviposition)	A	6.0	7.9	6.3	12.6	9.1	13.1	11.7	**	**	**	**	**	**
	B	I—VIII	VI—IX	IV—VIII	XI—XIV	VII—XI	XII—XIV	X—XIII	X—XIV	XII—XIII	XX—XIII	XII—XIII	IX—XIII	X—XIV

A = Average embryonic stage

B = The range of the embryonic stages found in the sample

* = The beginning of hatching

** = The embryos were found to be shrunk and distorted to a degree which did not allow exact classification, therefore the average embryonic stage was uncomputable.

↓ = The arrows in the squares—regarding the eggs still not moistened—show that although no examination was carried out, their embryonic stages may be considered as similar to those found among the eggs not moistened at all (the lowest row).

TABLE 4

Results of hatching of C. palaestinensis in groups of egg pods moistened at various intervals after oviposition at room temperature

	Days since oviposition / Days elapsed between oviposition and moistening	Number of nymphae hatched in each group moistened										Time of hatching not known	No. of nymphae hatched in the whole group	Mean duration of development in days	Mean percentage of hatching	Number of egg pods used for observations on hatching
		146–155	156–165	166–175	176–185	186–195	196–205	206–215	216–225	226–235	256–265*					
A. 1954/55	0 — 15					4	98	24	10	1			137	204.6	63.2	7
	30 — 50					23	25	1					49	196.5	55.2	5
	60 — 85					13	20	11	2				48	200.3	67.8	4
	145						26	1	12	2			41	209.9	62.9	2
	170								34	12			46	224.9	56.0	3
	210 — 220										15		15	256.8	34.1	2
Not moistened at all													0	—	0.0	1
B. 1955/56	0		150	115	8	1						43	317	164.6	84.5	12
	30	6	154	90	7	2						18	277	162.3	76.6	11
	85		19	34	60	10	5					11	139	177.3	73.3	8
	120				50	82	4					9	145	187.7	78.7	6
	150						44	1	7			9	61	202.3	36.3	6
	170							12	11			1	24	217.5	28.2	5
	198												0	—	0.0	1
Not moistened at all													0	—	0.0	5

* During the periods of 236—245 and 246—255 days no nymphs hatched in either season.

soil and for those moistened on the 30th and on the 85th day, but it was 210–256 days in the groups moistened later. However, during the second season the mean duration of the development in the same groups was 162–177 days and 187–217 days respectively.

TABLE 5

Dependence of the duration of development and the date of hatching upon the period elapsing between the oviposition and moistening of the eggs of Calliptamus palaestinensis at room temperature

a. 1954/55

Days elapsed between oviposition and moistening	Mean date of oviposition	Dispersion of the date of hatching	Mean date of hatching	Total duration of development in days*
0 — 15	24.IX.54	5.IV. — 12. V. 55	16.IV.55	204
30 — 50	24.IX.54	30.III — 18.IV.55	9.IV.55	197
60 — 85	25.IX.54	3.IV — 5. V. 55	14.IV.55	201
145	24.IX.54	18.IV. — 6. V. 55	23.IV.55	211
170	21.IX.54	1.V — 12. V. 55	4. V. 55	225
218	21.IX.54	4.VI — 6.VI. 55	5.VI. 55	257

b. 1955/56

Days elapsed between oviposition and moistening	Mean date of oviposition	Dispersion of the date of hatching	Mean date of hatching	Total duration of development in days*
0	7.XI.55	12.IV. — 10.V.56	20.IV.56	165
30	9.XI.55	10.IV. — 15.V.56	21.IV.56	164
85	25. X. 55	13.IV — 11.V.56	21.IV.56	179
120	26. X. 55	24.IV — 17.V.56	1. V. 56	188
150	26. X. 55	11.V — 2.VI.56	15. V. 56	202
170	15. X. 55	17.V — 23.V.56	20. V. 56	218

* As calculated from the difference between the mean date of oviposition and the mean date of hatching.

It is clear that the mean duration of the whole development increases markedly with a delay of moistening beyond 85 days (Tabels 4 and 5).

This mean duration of the total development (Table 5) was calculated by deduction of the mean date of oviposition from the mean date of hatching.

The same table (5a and 5b) shows clearly that the distribution of the hatching in time decreases markedly with the extension of the period between oviposition and moistening. However, it must be stressed that within the three latter groups of moistened pods, the hatchings occurred in the warmer season as compared with the earlier groups and therefore the influence of the increased temperature must also be taken into account (see conclusions).

B. Observations on egg development at 20°C

Observations on the development of the eggs kept at 20° ± 1°C and moistened t various times are cited in Table 6. This table shows that the eggs kept in dry ithout moistening reached the end of anatrepsis about the 70th day from oviposi- on. After this time the embryos which have already reached stage XI–XIV begin shrink and gradually die off in such a way that after 150 days there are no more iable eggs.

The eggs kept permanently in damp soil reach stages XIII–XIV after about 50 ays and the first embryos begin to turn round between the 70th and the 100th day fter oviposition. This phenomenon does not take place simultaneously in all mbryos; on the contrary, there is quite a long period of time during which turning ound may occur. There have been still viable embryos which did not begin to urn around even on the 160th day after oviposition and possibly later.

The rate of development of the eggs moistened on the 30th day after oviposition id not differ essentially from that of eggs kept permanently in damp soil. Eggs noistened on the 85th day lag slightly behind in the beginning of katatrepsis (the rst one turns around on about the 120th day). Under these conditions (85th day) art of the embryos still remained at the XIV stage even on the 160th day from viposition (similar to these kept permanently in damp soil as well as those moistened n the 30th day from oviposition).

The eggs moistened on the 120th and 150th day after oviposition showed first urnings around before the 30th day after moistening. The dispersion in time of the eginning of katatrepsis under these conditions was considerable and continued hrough the entire period of experimentation (up to 200 days after oviposition). n the group of eggs moistened on the 170th day after oviposition practically no mbryo turned around. In this group and in the group moistened on the 200th lay (not included in Table 6) no egg hatched.

Table 7 shows the influence of various periods of contact of the egg pods with he damp soil upon the percentage of hatching and the dispersion of hatching n time. The eggs kept permanently in damp soil hatched from the 116th till the 235th day after oviposition, and showed a high percentage of hatch (71%). Eggs noistened the 30th day hatched between the 116th and 185th day with 31% of natch. Those eggs moistened on the 85th day hatched between the 126th and the 205th day after oviposition. In the group of the egg pods moistened the 120th day, only one egg pod remained until hatching. Therefore, the results obtained (166–181 days as the period of hatching and 41.7% as the percentage of hatch) must be considered as not completely reliable. The egg pods moistened on the 150th day show a low percentage of hatch (9.6%) and consequently the data on the dispersion of hatching in time (196–215 days after oviposition) cannot be considered as reliable either.

TABLE 6

Successive stages of development of the eggs of Caliptamus palaestinensis in the season 1955/56 at 20°C. The results are arranged according to the groups of egg pods moistened at various times after oviposition. The figures included in each square are based upon the examination of 40 eggs taken out of 2–5 egg pods. The upper figure (A) in each square represents the average embryonic stage, the lower one (B) — the range of embryonic stages found among the sampled eggs.

Days between oviposition and moistening \ Days elapsed between oviposition and fixation of the eggs		10	15	20	30	40	50	70	100	120	140	160	180	200
0 (= Eggs kept permanently in damp soil)	A	1.1	1.8	5.2	9.3	11.2	13.9	13.9	13.6	15.3*	19.8	20.5		
	B	0—II	0—IV	III—VII	VI—XI	IX—XIII	XIII—XIV	XIII—XIV	XIII–XVIII	XIV–XXII	XIV—XXII	XIV—XXII		
Eggs moistened on the 30th day after oviposition	A	↓	↓	↓	↓	10.0		13.7	17.5	19.0*	20.2			
	B					VII—XII		XII—XIV	XIV—XXI	XIV–XXII	XIV–XXII			
Eggs moistened on the 85th day after oviposition	A	↓	↓	↓	↓	↓	↓	↓	14.0	14.2	14.3*	16.7		
	B								XIV	XIV—XVII	XIV–XXII	XIV–XXII		
Eggs moistened on the 120th day after oviposition	A	↓	↓	↓	↓	↓	↓	↓	↓	↓	12.4	17.7	*	
	B										XI—XIV	XIV—XXI		
Eggs moistened on the 150th day after oviposition	A	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	12.5	18.7	18.5*
	B											XII—XIII	XIV—XX	XIV–XXII
Eggs moistened on the 170th day after oviposition	A	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	**	**
	B												XI—XIV	XI–XVIII
Eggs moistened not at all (kept in dry soil since oviposition)	A	0.8	2.0	1.4	7.3	9.3	11.3	12.4	**	**	**	**	**	**
	B	0—I	0—V	I—II	II—XI	VIII–XII	XI—XII	XII—XIII	X—XIV	X—XIV	XI—XIV	X—XIV	X—XIV	unidentifable (shrunken)

A = Average embryonic stage.

B = The range of the embryonic stages found in the sample.

* = The beginning of hatching.

** = The embryos were found shrunk and distorted to a degree which did not allow exact examination, therefore the average embryonic stage was uncomputable.

↓ = The arrows in the squares — regarding the eggs still not moistened — show that although no examination was carried out, their embryonic stages may be considered as similar to those found among the eggs not moistened at all (— the lowest row).

TABLE 7

Results of hatching of C. palaestinensis in groups of egg pods moistened at various intervals after oviposition, at 20° C in the season 1955/56

Days since oviposition / Days elapsed betewen ovipositionand moistening	Number of nymphae hatched in each group												Time of hatching not known	No. of nymphae hatched in the whole group	Mean duration of development in days	Mean percentage of hatching	No, of egg pods used for observation on hatching
	116–125	126–135	136–145	146–155	156–165	166–175	176–185	186–195	196–205	206–215	216–225	226–235					
0	3	20	12	11	53	28	10	8	16	15	1	3	5	185	168.0	71.25	10
30	4	5	2	5	4	3	1						33	57	146.3	31.0	6
85		24	8	5	11	3	4	2	3				7	67	151.0	40.1	5
120						7	3							10	174.4	41.7	1
150									2	3				5	204.0	9.6	4
170														0	–	0	4
200–210														0	–	0	2
Not moistened at all														0	–	0	4

The results of preliminary experiments carried out during the season 54/55 (not reported here) agreed well with the results of season 55/56 described above.

C. Observations on egg development at 27°C

The results of the experiments at the temperature of 27°C are cited in Table 8. As only one egg hatched, results could be obtained only by checking the stages of the embryos in the various groups of moistening.

The eggs kept permanently in dry soil reached the end of anatrepsis (stage X–XIII) within 40 days. From the 50th day onwards the eggs began to shrink and no embryo passed katatrepsis. The last, apparently viable, eggs were found on the 120th day after oviposition.

The eggs kept permanently in damp soil 30 days after oviposition already included some embryos which have reached the end of anatrepsis. On the other hand, some embryos of earlier stages were found even at the 200th day after oviposition. No embryo passed katatrepsis and consequently none hatched in this group. Almost similar results were obtained from the groups of the egg pods moistened on the 30th, 85th and 120th day after oviposition. Three embryos only (out of some 400) pass the katatrepsis in the 30th day moistening group and one of them hatched. One embryo only turned around on the group moistened on the 85th day and no embryo did so in the 120th day group. In all the above groups, the embryos appeared viable.

In the 150th and 170th day groups, however, the embryo remained shrunken after the moistening and no embryo resumed its development.

D. Observations on eggs transferred from room temperature to 29°C

Two egg pods laid on 24th and 26th of September 1954 and moistened on the 31st day afterwards, were transferred from room temperature to 29 ± 1°C on the 19th of February (146–148 days since oviposition). The eggs at the time of the transfer contained large embryos, some of which were of the XX stage. These eggs thus transferred hatched some 6 to 9 days afterwards, whereas the controls kept in damp soil since the 30th day after oviposition at room temperature hatched in April (Table 5a) i. e. some 40 days later (average).

CONCLUSIONS

1. *Life cycle*

The hatching in the field seems to take place in April-May, as the young hoppers were found in the field in May-June. The adults appear from June to February, the last has been found at the beginning of March.

The grasshoppers caught in the field laid their eggs in the laboratory from the middle of August or from the beginning of September up to November or even December. The eggs kept permanently in damp soil at room temperature or transferred

TABLE 8

Successive stages of development of the eggs of Calliptamus palaestinensis in the seasson 1955/56 at 27°C. The results are arranged according to the groups of egg pods moistened ot various intervals after oviposition. The figures included in each square are based upon the examination of 40 eggs taken out of 2–5 egg pods. The upper figures (A) in each square represents the average embryonic stage, the lower one (B) — the range of embryonic stages found among the sampled eggs.

Days elapsed between oviposition and fixation of the eggs / Days elapsed between oviposition and moistening		10	15	20	30	40	50	70	100	120	140	160	180	200
0 (= Eggs kept permanently in the damp soil)	A	4.9	6.8	10.0	12.5	10.5	10.7	13.0	13.2	13.4	12.5	11.9	13.0	10.5
	B	I—VIII	IV—IX	VII—XI	X—XIV	V—XIV	V—XIV	XI—XIV	XII—XIV	XIII—XIV	X—XIII	VII—XIV	XIII	VIII—XIV
Eggs moistened on the 30th day after oviposition	A	↓	↓	↓	↓	11.2		12.6	12.6	13.1	12.8	13.8	14.8	12.8
	B					IX—XIII		XII—XIV	XII—XIV	XI—XIV	XI—XIV	XI—XXI	XIII-XXII	X—XIV
Eggs moistened on the 85th day after oviposition	A	↓	↓	↓	↓	↓	↓	↓	12.2	12.2	12.1	12.5	13.0	12.3
	B								XII—XIII	XII—XIII	X—XIII	XII—XIII	XII—XX	XII—XIII
Eggs moistened on the 120th day after oviposition	A	↓	↓	↓	↓	↓	↓	↓	↓	↓	12.8	13.3	13.0	13.5
	B										XII—XIV	XII—XIV	XII—XIV	XIII—XIV
Eggs moistened on the 150th day after oviposition	A	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	**	**	**
	B											XI—XII	X—XII	XI—XIII
Eggs moistened on the 170th day after oviposition	A	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	**	**
	B												VIII—XI	IX—XI[1]
Eggs not moistened at all (kept in dry soil since oviposition)	A	2.5	5.2	10.6	8.3	11.3	**	**	**	**	**	**	**	**
	B	I—V	I—VIII	VIII—XII	VII—X	X—XIII	VIII—XIII	X—XIII	X—XII	X—XII	IX—XIV	X—XIII	+	+

A = Average embryonic stage.
B = The range of the embryonic stages found in the sample.
** = The embryos were found to be shrunken and distorted to a degree which did not allow exact indentification, therefore the average embryonic stage was uncomputable.
\+ = The embryos were found shrunk and distorted to a degree which did not allow identification at all.
↓ = The arrows in the squares — regarding eggs still not moistened — show that although no examination was carried out, their embryonic stages may be considered as similar to those found among the eggs not moistened at all (— the lowest row).

to it on the 30th and 85th day after oviposition hatched from the end of March until the middle of May (see Table 5).

The mean duration of development of *Calliptamus* nymphae as observed in the laboratory at nearly 30°C (from hatching up to last moult) was found as 39 ± 3.7 days for males and 50 ± 8 days for females. Statistical analysis shows that this is a significant difference.

In the laboratory, the last grasshoppers died in the first days of January.

2. *Egg development*

The egg pods are laid in the laboratory only into dry soil.They are surrounded by a very hard membranous envelope strengthened by the soil particles imbedded in it. When moistened, the envelope becomes loose and soft.

The conclusions regarding the influence of temperature and contact moisture upon the development of the egg of *Calliptamus palaestinensis* will be considered under three separate headings concerning three phases of embryonic development: A. predormant period, B. a period of interrupted morphological differentiation (diapause and/or dormancy), and C. postdormant period.

A. *Predormant period* (from stages 0 up to XI–XIV)

The development of eggs starts within a few days after oviposition so that after 10 days the embryos are already in stages I–VIII at 27°C and O–II at 20°C.

Eggs kept permanently in dry soil were compared with eggs kept continuously in damp soil regarding the rate of their embryonic development during the first 40 days after oviposition at 20°C.

The same comparisons were carried out with eggs kept in dry and moist soil at 27°C. The statistical analysis (two sided t-test for paired variates) of the mean embryonic stages has shown that at both temperatures there is no significant difference in the rate of development of the eggs kept in dry soil during the first 40 days since oviposition as compared with those kept in damp soil.

for 20°C	t_4	= 2.20	$0.10 > p > 0.08$
for 27°C	t_4	= 1.45	$0.30 > p > 0.20$

The above conclusion allowed us to compare the rate of development of eggs kept at 20°C with those kept at 27°C during the first 40 days of their development regardless of the humidity condition of the soil at which the eggs were kept. The observations of the eggs kept at all conditions of soil were considered as an entire group for each period (10, 15, 20, 30 and 40 days after oviposition) and compared for both temperatures of 20°C and 27°C (Table 9).

TABLE 9

Influence of temperature upon the rate of development of the embryos of C. palaestinensis during anatrepsis. The figures show the mean stages of development calculated from the average embryonic stages found in groups of egg pods kept permanently in damp and dry soils

Temperature in °C \ Days elapsed between oviposition and fixation of the eggs	10	15	20	30	40
20	0.95	1.9	3.3	8.3	10.25
27	3.70	6.0	10.3	10.4	10.9

The statistical analysis of the above results (two sided t-test for paired variates) has shown hat the influence of the temperature is significant ($t_4 = 3.1$ $p < 0.05$).

The periods of time elapsed between oviposition and certain enbryonic stages at he temperatures 20°C and 27°C were compared on the assumption that the influence f the temperature may be expressed by means of the Blunck-Bodenheimer's hyperbolic urve (T—C) t=a constant (where T = temperature, C = the threshold of development and t = time).

Two mean embryonic stages (3.7 and 10.3) (Table 9) were chosen.

We have used 10 days as the period needed for development until the mean stage .7 at 27°C and 21 days for the same stage at 20°C. 20 days were used for the same urpose for the mean stage 10.3 at 27°C, and 40 days as a period needed for development at 20°C. The threshold of development was calculated as 13.6 and 13°C espectively.

The anatrepsis is concluded at 27°C within 40 days (Table 8), at 20°C within 0–70 days (Table 6) and at room temperature within 50 days (Table 3). The mean emperature of the room was at the time of anatrepsis 26.4°C for October, 20.5°C for lovember and 18.1°C for December (Table 2b).

B. *Diapause* (stages XI–XIV)

The embryo, after reaching stages XI–XIV, enters into a particular period of evelopment wherein no further external morphological differentiation takes place. uring the earlier period of diapause the embryo seems to be ready morphologically r katatrepsis but only during the latter part of this period it becomes ready physiolocally for turning around. The period of diapause continues until the beginning of atatrepsis, which takes place only at certain conditions of temperature and soil umidity.

The beginning of the "dormant" period in our experiments takes place some 40 70 days after oviposition depending on the emperature. The eggs kept permanently dry soil are unable to resume their development if not moistened. However, many gs kept continually in damp soil as well as those transferred to damp soil from dry

soil resumed their development even when kept for 150–218 days in dry soil befo moistening.

The shortest "dormant" period occurs with the eggs kept continuously in dan soil or moistened within the anatreptic period.

Together with the delay in the time of moistening the "dormant" period becom more and more extended. This extension, however, seems to increase at the exper of that part of the "dormant" period during which the embryo is ready for katatrep physiologically as well as morphologically, but starts it only when moistened.

The influence of temperature is negative at 27°C, when only 4 embryos out of mo than 1000 showed katatrep is under all conditions of soil humidity. The difference the way of development of the eggs at 20°C in comparison with that at room temper ture is prominent and is expressed in the very wide distribution in time of katatrep at 20°C. The synchronic katatrepsis observed in the eggs kept in the room may be attrib ted to the influence of the low temperatures (especially at night) prevailing in wint

Thus two factors seem to be essential for the ending of the period of dormanc the contact with water from the damp soil surrounding the egg pod and comparati low temperatures.

C. *Postdormant period* (from stage XV until hatching)

The embryos physiologically ready for katatrepsis start turning around a having imbibed enough water for this purpose. The development during the p dormant period is smooth and the hatching takes places without difficulty. T postdormant period (i. e. the time from the beginning of katatrepsis until hatchi clearly depends on external temperature.

Table 10 shows the duration of development between moistening and hatchi with respect to groups of eggs moistened on various dates at room temperatur We used for this purpose egg pods which were moistened when, according to o assumption, the embryos were physiologically as well as morphologically ready imbibe water and to resume their development.

TABLE 10

Effect of delay in the date of moistening upon the acceleration of the postdormant development C. palaestinensis eggs kept at room temperature.

Season	The mean date of moistening (in brackets the range)	Days elapsed between oviposition and moistening	The mean age of eggs at hatching in days	Days passed between moistening and the mean age of eggs at hatching	No. of egg po used
1954/55	16.II.1955 (13.II—19.II.1955	145	209.9	64.9	2
	10.III.1955 (9.III—11.III.1955)	170	224.9	54.9	3
	27.IV.1955	218	256.8	38.8	1
1955/56	15.II.1956	120	191.1	71.1	4
	11.III.1956	120	180.4	60.4	2
	14.III.1956	150	202.3	52.3	6
	2.IV.1956	170	217.5	47.5	5

From this table it is evident that when the moistening occured later in the season the temperatures then being higher) the duration of the development between moistening and hatching clearly decreased.

An additional proof of the influence of temperature upon the rate of postdormant development was produced by the transfer of the eggs fron the room into a thermostat of 29°C (see page 146). The eggs so transferred hatched after 6–9 days. Those, however, which remained at room temperature hatched after 40 days (average).

DISCUSSION

. *Oviposition and egg pod*

The eggs of Acrididae laid into damp soil such as those of the tropical species *ocusta migratoria migratorioides*, *Schistocerca gregaria* and *Nomadacris septemfasiata* usually develop without interruption. Their development takes a few weeks nly, provided that the temperature of the environment is sufficiently high. In these ases, the imbibition of water by the eggs begins in earlier embryological stages Shulov 1952a, Shulov and Pener 1959, Shulov and Pener in preparation). In eggs of ypically temperate region Acrididae, an interruption of development takes place even hen laid into damp soil (Richards and Waloff 1954).

Those eggs, which are laid into dry soil develop slowly and do not imbibe water uring the first stages of their development. Some even loose water during this period *Dociostaurus*). The water is imbibed during the later parts of the embryonic development when the embryo is ready morphologically as well as physiologically for katepsis (Bodenheimer and Shulov 1951). Only one exemption is known: that of *methis pulchripennis* in which the eggs develop without addition of water (Shulov 952b).

The eggs of *Calliptamus palaestinensis* are laid always into dry soil, as the females now clear preference for it. However, the eggs of this grasshopper start developing hether in contact with water or with dry soil. In this they are similar to the eggs of *ocustana pardalina* (Matthée 1951).

The envelope of the egg pod of *Calliptamus palaestinensis* is hard and tight and pparently prevents the eggs from dessication in dry soil. In this respect the egg pods re similar to those of *Dociostaurus* (Bodenheimer and Shulov 1951) and *Tmethis* Shulov 1952b). The plug of the egg pod is small, variable in shape and does not resent any air space or lid.

. *Stages of embryonic development*

lifer (1932) was the first to describe the morphological changes of the embryo of *Melanoplus* through day by day pictures. Later investigators have made endeavours o set up arbitrary stages of development in which stress was laid upon the major

morphological changes (Steele 1941, Matthée 1951, Bodenheimer and Shulov 195 Shulov and Pener 1959). In the present paper, the denomination of the stages s similar to that employed by Bodenheimer and Shulov (1951) for *Dociostaur maroccanus* (with slight changes), as the morphological differentiation of the embry of both species is similar.

The embryonic development of *C. palaestinensis* is discussed here under thr headings:

A. Predormant period including anatrepsis (Stages 0 to XI—XIV),

B. Dormant period or diapause (Stages XI—XIV),

C. Postdormant period including katatrepsis (Stages XV—XXII).

A. *Predormant period*

There are some Acrididae such as *Dociostaurus* and *Austroicetes* whose egg start their development only several weeks after oviposition. The development of th eggs of *Calliptamus palaestinensis* starts within 10 days under all conditions of ou experiments. The eggs develop when kept either in damp or dry soil and seem t reach the same stages within equal periods of time when kept at the same temperatu Thus the eggs of this *Calliptamus* species differ from the eggs of *Locustana pardali* which develop more quickly when kept in damp soil in comparison to those kept dry soil (Matthée 1951).

The development of the eggs of *C. palaestinensis* during the period of anatrepsis significantly more rapid at higher temperatures (at 27°C as opposed to 20°C). Th influence of temperature was calculated by means of the Blunck–Bodenheime hyperbolic curve. It was found that the threshold of development for the perio from oviposition to average embryonic stage "3.7" is 13.6°C with 140 day-degrees temperature and for the period from the oviposition to the average stage "10.3" 13°C with 280 day-degrees.

B. *Dormant period* (Diapause) (Stages XI—XIV)

The concept, diapause, in insects embraces a very wide range of phenomena whic are caused by many intrinsic as well as extrinsic factors (Lees 1955). We use th word diapause in the sense applied to Acrididae which includes the interruption o development found in *Melanoplus differentialis* as well as that found in *Dociostaurus marrocanus*.

The acridian eggs pass this dormant period usually in the stage which correspond in general with our XIV stage (end of anatrepsis period) (Shulov 1953).

During this period the eggs of some species lose part of their content of water, bu this loss may not exceed a certain maximum; otherwise the eggs dessicate and die

However, an embryo which appears shrunken and dessicated is still able to develop the period of "dessication" does not extend beyond a point which might be individual or each species. Under natural conditions, this period continues with most of the grasshoppers for several months, but in extreme cases it can continue for more than two years (Matthée 1951).

In the case of *Dociostaurus* (Bodenheimer and Shulov 1951), this interruption of development is stable and the embryo is unable to resume it, whether through thermal or chemical stimulation, at least not during the first period of diapause (stage B in *Dociostaurus* accroding to Bodenheimer and Shulov 1951). During the second period (stage C, ibid), the eggs are able to imbibe water and resume their development in a short time.

In the case of *Melanoplus differentialis* (Slifer 1932) it seems that the interruption of development can be avoided by influence of low temperatures. Richards and Valoff (1954) have shown that if the eggs of *Chorthippus brunneus* are kept at 25°C for one year, they do not hatch but die. However, when kept at 0°C for various periods and later transferred to 25°C, the eggs hatched as follows: when kept for two weeks — no hatch; four weeks — poor hatch; six weeks — full hatch. Steele (1941) has shown that the eggs of *Austroicetes*, which were collected in the field in their diapreptic period, developed when placed into 25°C a little further, but did not hatch. Bodenheimer and Shulov (1950) working with the eggs of the northern race of *Locusta migratoria* has shown that at 30°C they remain in diapause at least 3 months and resume their development only when kept for one month at 0—6°C. Those eggs which did not pass the cold conditioning showed a very low percentage of hatching (1—2.5%).

We have been unable to find a clear cut division into two periods in the eggs of *Calliptamus*, as found in *Dociostaurus*. Although the imbibition of water presents a necessary condition for the further development of *Calliptamus* eggs which were kept in dry soil, they are able to remain viable dormant for 5 to 6 months and even more at room temperature. Those eggs, however, which were moistened during the predormant period show the shortest extent of the entire embryonic development in comparison with the eggs moistened later at room temperature.

On the other hand, it seems quite probable that the eggs of *C. palaestinensis* need a comparatively low temperature in order to be able to continue their development. When kept continuously at 27°C, practically no embryo passed katatrepsis in all moistened groups; when kept continuously at 20°C the katatrepsis was passed, but was widely distributed in time, and when kept at room temperature, which was quite low at night (during January and February), the eggs entered uniformly into katatrepsis (in February).

The presence of eggs with a short diapause together with those with a longer one was found by us in *Calliptamus palaestinensis* at 20°C, in contradiction to the eggs of the same species kept at room temperature which enter and leave the diapause

almost simultaneously, bring to mind the finding of Matthée (1951). He described the presence of diapause and non-diapause eggs in the same egg-pods of *Locustana paradalina*. One wonders whether, had Matthée experimented with eggs at a lower temperature, would he not have found that the percentage of the diapause eggs is much lower and the dispersal in time of those eggs which entered katatrepsis less prominent.

The resumption of the morphological differentiation of the embryo and the further development of the egg of *C. palaestinensis* are thus connected primarily with temperature and moisture.

C. *Postdormant period*

Katatrepsis is defined as a succession of stages of the steadily growing embryo which bring it in position so that its head is turned to the non-micropylar end of the egg.

This is the morphological aspect, while from the physiological one it is usually connected with a previous imbibition of water by the egg. In non-diapause eggs, katatrepsis takes place immediately after anatrepsis, but it can be delayed by controlling the water supply during the first days of development (*Schistocerca*, *Locusta*, *Nomadacris*) and later resumed by the addition of water (Shulov & Pener in preparation). In diapause-eggs, the beginning of katatrepsis may occur after many months upon addition of water.

The rate of katatrepsis and of further development until hatching is clearly influenced by temperature as has been shown in *Dociostaurus* (Bodenheimer and Shulov 1951). Experiments carried out on *Calliptamus palaestinensis* showed that some time may elapse between the date of moistening and the beginning of katatrepsis.

The rate of development of *C. palaestinensis* during the postdormant period can be considerably increased by the influence of temperature.

3. *Annual cycle of C. palaestinensis in nature*

In Israel the rains start usually in November, and the coldest period continues from January to February–March. In April, the temperature rise, causing abundant ephemeral green cover (herbs) during April–May.

The main oviposition of *C. palaestinensis* occurs before the rains. The eggs can thus develop slowly either in dry soil or when moistened shortly after oviposition. Thus the period of main hatching occurs in April–May when sufficient green food is available. The nymphae and later the adult grasshoppers are able to find enough food for themselves during the late spring and summer and reach sexual maturity in the beginning of autumn.

The development of the eggs of *C. palaestinensis* presents an example of a monovoltine grasshopper egg which is ecologically adjacent to those typical of the temperate

d climates characterised by stable diapause (*Dociostaurus*) and those fitted for der climates which evolutionally become connected with low temperature during diapause (*Chorthipus brunneus*).

ACKNOWLEDGMENTS

e authors express their sincere thanks to Dr. J. Wahrman and Mr. L. Fishelsohn having kindly placed their collections at their disposal and to Mr. P. Amitai for paration of the drawings.

REFERENCES

ANDREWARTHA, H. G., 1945, Some differences in the physiology and ecology of locusts and grasshoppers, *Bull. ent. Res.*, **35**, 379—389.

BEI-BIENKO, G. J. AND MISHCHENKO, L. L., 1951, *Saranchevye fauny S.S.S.R. i sopredelnych stran* (*Acridoidea from the fauna of U.S.S.R. and of adjacent countries*), **1**, Academy of Sciences of U.S.S.R., Moscow, 378p.

BODENHEIMER, F. S., 1935, *Animal Life in Palestine*, Jerusalem, 506p.

BODENHEIMER, F. S., 1937, Prodromus Faunae Palaestinae, *Mem. Inst. Egypte*, **33**, 1—286.

BODENHEIMER, F. S. AND SHULOV, A., 1951, Egg development and diapause in the Moroccan Locust (*Dociostaurus maroccanus* Thnb), *Bull. Res. Counc. of Israel*, **1**, 59—75.

CHAPMAN, R. F. AND ROBERTSON, I. A. D., 1958, The egg pods of some tropical African grasshoppers, *J. ent. Soc. S. Afr.*, **21**, 85—112.

HUNTER-JONES, P., 1956, *Instructions for Rearing and Breeding Locusts in the Laboratory*, London, Anti-Locust Research Centre, 9p.

JACHIMOVICH, L. A., 1950, Smena trebowanyi k uslovijam embryonalnovo razvitija asiatskoj saranchi, (The changes in requirements for the conditions of the embryonic development of the Asiatic locust) (in Russian), *Dokl. Akad. Nauk. U.S.S.R.*, **73**, 1105—1108.

LEES, A. D., 1955, *The Physiology of Diapause in Arthropods*, Cambridge, University Press, 151p.

MATTHÉE, J. J., 1951, The structure and physiology of the egg of *Locustana pardalina* (Walk), *Sci. Bull. Dep. Agric. S. Afr.*, 346, 1–83.

RAMME, W., 1951, Zur Systematic Faunistic und Biologie der Orthopteren von Sudost Europa und Vorderasien, *Mitt. Zool. Mus. Berlin*, **27**, 1—431.

RICHARDS, O. W. AND WALOFF, N., 1954, Studies on the biology and population dynamics of British grasshoppers, *Anti-Locust Bull.*, 17, 1—182.

SHULOV, A., 1952a, The development of eggs of *Schistocerca gregaria* (Forskàl) in relation to water, *Bull. ent. Res.*, **43**, 469—476.

SHULOV, A., 1952b, Observations on the behaviour and egg development of *Tmethis pulchripennis asiaticus* Uv., *Bull. Res. Counc. of Israel*, **2**, 249—254.

SHULOV, A., 1953, The role of water in the eggs of Acrididae, *Proc. XIV Internat. Congress Zoology*, Copenhagen, 1953 (1956), 395—401.

SHULOV, A. AND PENER, M. P., 1959, A contribution to knowledge of the development of the egg of *Locusta migratoria migratorioides* (R & F), *Locusta*, 6, 73—88.

17. SLIFER, E. H., 1932, Insect development. IV. External morphology of grasshopper embryos
known age and with a known temperature history, *J. Morph.*, **53**, 1—21.

18. STEELE, H. V., 1941, Some observations on the embryonic development of *Austroicetes crucia*
Sauss. (Acrididae) in the field, *Trans. R. Soc. S. Aust.*, **65**, 329—332.

19. UVAROV, B. P., 1928, *Locusts and Grasshoppers*, London, The Imperial Bureau of Entomolo
352p.

HE BENTHONIC AMPHIPODA OF THE MEDITERRANEAN COAST OF ISRAEL

I. NOTES ON THE GEOGRAPHICAL DISTRIBUTION

E. GOTTLIEB
Sea Fisheries Research Station, Haifa

ABSTRACT

The majority of species of Amphipoda reported from the eastern Mediterranean are also on record from the western and central part of this sea, many of them being Atlantic (Lusitanian region) and North Sea fauna. The Atlantic-Mediterranean elements are well represented in the amphipod fauna of the Mediterranean coast of Israel, which also includes cold water species known from boreal and arctic regions. This fauna includes also tropical elements, one of which has penetrated from the Red Sea into the eastern Mediterranean through the Suez Canal.

A list of all the identified species and their geographical distribution is given at the end of this paper.

INTRODUCTION

e Amphipoda have a wide geographical distribution and are quantitatively well presented in most seas, forming an important element of the benthonic fauna. They very abundant among the bottom invertebrates of the Mediterranean continental elf of Israel forming an important item in the diet of the demersal Teleostei.

The amphipod fauna of the eastern Mediterranean has been previously studied ng the Egyptian coast near Alexandria by Schellenberg (1936)*; many species he ed have also been found in the course of this study.

The penetration of Amphipoda from the Red Sea to the eastern Mediterranean ough the Suez Canal was first noticed after the study of the collection made by Cambridge Expedition to the Suez Canal (Schellenberg, 1928). This was later nfirmed by the studies of the amphipods collected along the coast of Eritrea uffo, 1938).

This paper is the first of a two-part study on the amphipods in the Mediterranean ters of Israel. The second part, dealing with the life history and ecology of a few ecies abundant in the Caesarea rock-pools, will be published at a later date. This dy of the geographical distribution of Amphipoda is based on material collected ng the continental shelf of Israel in the course of the survey of the benthos or-

* All references to amphipods from the region of Alexandria, Egypt are taken from Schellenberg (36).

ceived January 19, 1960

ganisms carried out on board a research vessel, during the years 1946–1950. Samples c both sandy and muddy sea bottom were taken at depths of 10, 30, 50 and 75 fathom along profiles perpendicular to the coast line at localities mentioned in Table I (se also sea chart in Figure 1). Additional material collected from the Caesarea rock-poo during the years 1951–1952, was used to exemplify the distribution of the littor species, as well as to study their ecology.

TABLE I

Distribution of Amphipoda along the coast of Isreal, from North to South, in the Mediterranea
(for localities mentioned see sea chart in Figure 1)

	Nahariya	Atlit	Caesarea	Natanya	Herzliya	Tel-Aviv	N. Rubin	N. Yunis	Ascalon	Ga
Ampelisca spp.	+	+	+	+	+	+	+	+	+	+
Haploops della-vallei	+	+	+	+	+	+	+	+	+	
Bathyporeia cf. guilliamsoniana		+	+	+						
Urothoe grimaldii	+	+	+					+	+	+
Harpinia cf. pectinata		+	+	+	+	+	+	+	+	+
Leucothoe lilljeborgi	+	+	+	+		+				+
Monoculodes subnudus	+					+				
Nototropis swammerdami	+	+		+						
Eriopisa elongata	+	+	+	+	+	+	+	+	+	
Maera grossimana	+	+							+	
Microdeutopus gryllotalpa	+	+	+			+	+	+	+	
Pseudoprotella phasma	+	+	+	+						
Phtisica marina	+	+	+					+		

The geographical distribution of Amphipoda species along the coast of Israel compared with their distribution in the Mediterarnean Sea and other regions a described by Chevreux and Fage (1925). The latter publication forms basic referen to the zoogeography of amphipods in this study while other more recent papers a referred to in the text.

AMPHIPODA IN THE LITTORAL ROCK-POOLS

The most conspicuous family of amphipods in the Caesarea rock-pools is the famil Amphithoidae which is widely distributed in all climatic zones. Two species of th family are present in our waters: *Cymadusa filosa* and *Amphithoe ramondi.** *C. filos* lives in temperate and tropical zones of the Atlantic-Mediterranean region; *A. ramondi* is a circumtropical species. Both these species were recorded in Italian wate by Ruffo (1938, 1946). However, only *A. ramondi* was reported from the Egyptia coast. Both species were also recorded from Eilat, Gulf of Akaba and from th Eritrean waters near Massawa by Ruffo (1938, 1959).

* The life history of *C. filosa* and *A. ramondi* in the Caesarea rock-pools will be described in th second part of this study.

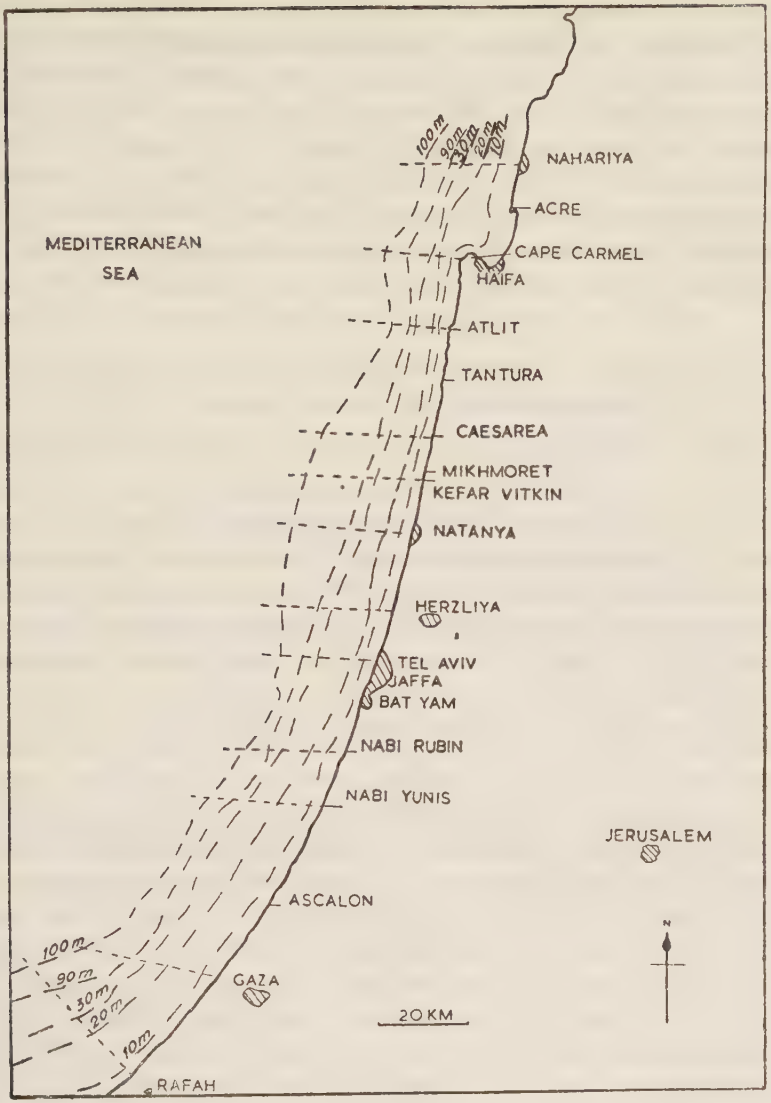


Figure 1.
Chart of the sea of the Israel coast showing the lines along which the collections were made.

The family Talitridae is also abundant in the Caesarea rock-pools. *Talitrus saltator*, very common along the Mediterranean coast of Israel, has adapted itself to a terrestrial mode of life, burrowing into the wet sand near the water line. It is an inhabitant of the Lusitanian region in the Atlantic Ocean, appearing as far north as the Norwegian coast, and is present in the entire western Mediterranean.

The most abundant species found among the algae of the rock-pools is *Hyale schmidti* (Talitridae). This species is abundant in the Atlantic-Mediterranean region and also recorded in the Egyptian waters; it is also present in the waters of Japan and the Red Sea (Ruffo, 1950). Another species, *H. prevosti*, which is also found in the Caesarea rock-pools, has a wide distribution in the Atlantic where it has been found along the coasts of Europe, Africa and America, as well as in the Mediterranean along the Algerian, Italian and Egyptian coasts (Ruffo, 1938). It is very abundant in the lagoons of Venice (Soika, 1949). At the Egyptian coast near Alexandria it has been collected at a depth of up to 100 metres which is very unusual for this littoral species.

The genus *Hyale* has a world-wide distribution; Ruffo (1950) presents a list of 42 species, 13 of which are inhabitants of the Atlantic region, 5 of the Atlantic-Mediterranean region, 2 endemic in the Mediterranean, and 22 are found in the Indo-Pacific region.

Next in the order of abundancy in the Caesarea rock-pools is the family Gammaridae represented by *Elasmopus pocillimanus* and *E. pectenicrus*. *E. pocillimanus* has a wide distribution in the Pacific and Atlantic, being present along the coasts of Europe, Africa and America and in the Mediterranean along the Algerian, Tunisian, French, Italian and Egyptian coasts. It has been also recorded from the Red Sea near the Eritrean coast (Ruffo, 1938).

E. pectenicrus, which occurs in large numbers in our rock-pools, has been found in the following areas: Hawaii, New Guinea, Ceylon, Brazil, Porto Rico, Sierra Leone, Belgian Congo and South Africa. This species has not been found in the western Mediterranean, but was noted in the eastern Mediterranean (Ruffo, 1959). Before that, it had been identified among the Suez Canal fauna (near Port Said) by Schellenberg (1928) and somewhat later it was also recorded in the harbour of Alexandria. Schellenberg (1936) writes: "it occurs frequently in the Indian Ocean between the sea plants growing on bodies of ships, buildings in the harbours and at the bottom. Their habitat makes them very fitted for being transported.Transportation from the Indian Ocean into the Mediterranean is very probable." Although the species has not yet been found in the Gulf of Suez or in the Gulf of Eilat, from its presence elsewhere in the Red Sea and in the Suez Canal, it may be inferred that it has penetrated from the Indo-Pacific region into the eastern Mediterranean (Ruffo, 1938). At present, this species is known not only from the Caesarea rock-pools but it has also been identified among the fouling organisms on ships in Haifa harbour. There is no record of its distribution further north in the eastern Mediterranean.

The family Caprellidae is represented in the Caesarea rock-pools by *Caprella hirsuta* and *C. acanthifera*. Both species are known from the western Mediterranean and the Adriatic Sea (Fiorencis, 1940). Among the littoral algae of French waters in the western Mediterranean, appear species different from those mentioned in this study.

Another species of the rock-pools of Caesarea, *Microdeutopus gryllotalpa* (family Aoridae), has a wide distribution extending along the Atlantic coast of America and Europe as far north as Norway, and is present also in the Mediterranean. *M. gryllotalpa* lives in the lagoons of Venice (Soika, 1949) and in the Egyptian coast near Alexandria.

Lysianassa ceratina (family Lysianassidae) appears in the Lusitanian and Mauretanian regions of the Atlantic and in the Indian Ocean; it has been recorded from the Red Sea by Ruffo (1938). In the Mediterranean, it is present along the coasts of Algeria, Tunisia and Greece as well as in the Adriatic Sea (Ruffo, 1936). This species is common in the Caesarea rock-pools particularly during the summer.

AMPHIPODA OF THE CONTINENTAL SHELF

The description of benthonic communities of the continental shelf of Israel is based on the material collected by Wirszubski (1953)* at the depths of 10–75 fathoms. In this paper a few species of amphipods (examined and identified by this author) were mentioned as characteristic for the animal bottom communities. A more detailed examination of this material revealed the existence of a large number of species which are included in the list of Amphipoda at the end of this paper, dealing with their geographic distribution.

The bathymetric and geographical distribution of the identified species common on the continental shelf, is given in Tables I and II. From the presence of several

TABLE II

Bathymetric distribution of Amphipoda from the Israel continental shelf in the Mediterranean, based on the material collected during the benthos survey (1946–1950)

Species	Depth in metres			
	18	54	90	135
Ampelisca spp.	+	+	+	+
Haploops della-vallei		+	+	+
Pathyporeia cf. guilliamsoniana	+	+	+	
Urothoe grimaldii	+	+	+	+
Harpinia cf. pectinata		+	+	+
Leucothoe lilljeborgi	+	+	+	
Monoculodes subnudus	+	+	+	
Lototropis swammerdami	+			
Oriopisa elongata		+	+	+
Maera grossimana	+			
Microdeutopus gryllotalpa	+	+	+	+
Pseudoprotella phasma	+	+		
Phtisica marina	+	+		

* After this preliminary study, the material was forwarded to Prof. S. Ruffo, Museum of Natural History in Verona (Italy) for further examination.

species at stations situated along a section of the coast (Table I), no definite conclu sions can be drawn as to the limits of their distribution. However, from the bathy metric distribution (Table II) it may be concluded that while some species are limite to depths of 18–54 metres, most of them extend into deeper waters.

The commonest species of the continental shelf belong to the family Ampeliscida represented by *Ampelisca brevicornis*, *A. tenuicornis*, *A. sarsi*, *Haploops della-vallei.*

A. brevicornis is well known along the Atlantic-European coast and very abundan in the North Sea where, according to Blegvad (1930), its yearly production exceed several times the original stock. Its ecology in the Irish Sea, at the southern coas of the Isle of Man, was described by Jones (1947). It has also been found in th Indian Ocean (Barnard, 1937) and in the Pacific. In the Mediterranean this specie is known from the coasts of France, Italy, Algeria and has also been recorded fro Egypt by Schellenberg (1936). The second species, *A. tenuicornis*, has a simila geographic distribution in the Atlantic-Mediterranean region and is also on recor from the Indian Ocean (Barnard, 1937). *A. sarsi* is a rare species known from th Atlantic and the Mediterranean Sea (Ruffo, 1936).

Haploops della-vallei, which is endemic in the Mediterranean, has been describ from the Gulf of Naples, Italy, and from the continental shelf of Israel where inhabits tubes made from sticky particles of sand; similar to those of *H. tubic* in the northern waters of the Arctic, Atlantic and Pacific Oceans.

The family Gammaridae is represented in waters of Israel by several specie the most abundant of which are *Echinogammarus olivii* taken from shallow water and *Eriopisa elongata* common in deeper waters of the continental shelf. The latte species is of Atlantic-Mediterranean distribution, reported from the eastern Mediter ranean, near Alexandria. Amphipods of the family Gammaridae other than thos present in the rock-pools, are rarely seen. Those which do occur are included in th list of species at the end of this paper.

Gammarus locusta, which is known for its wide distribution in saline and brackis waters in the Arctic, Pacific and Atlantic Oceans as well as in the Mediterranean Se (European and North African coasts), has been found only once in Haifa Bay on sandy bottom in shallow water. This species was not included in the collection o Amphipoda from the Egyptian waters near Alexandria made by Steuer. This fac drew the attention of Schellenberg (1936) who wrote: "it is striking that *G. locusta* an euryhaline and eurythermal species which is common on the whole Atlanti coast of Europe as well as in the Mediterranean, is missing. It seems to be replace at Alexandria by *Hyale schmidti*, the only marine amphipod appearing in grea quantities between the algae." It is worth mentioning that *G. locusta* has not bee found during our extensive survey of the Caesarea rock-pools, but its existenc in the eastern Mediterranean has been demonstrated without a doubt.

The family Haustoridae is represented on the continental shelf of Israel by *Bathyporeia guilliamsoniana* which occurs on sandy and muddy bottoms. This species, of Atlantic-Mediterranean distribution, has been previously recorded from the Egyptian coast. Watkin (1939), who made a revision of the genus *Bathyporeia*, states that it extends into comparatively deep waters off the Atlantic coast of Europe but is also very abundant in the intertidal sands. Watkin mentions also that *B. guilliamsoniana* is very abundant in the North Sea, where it forms an important item in the diet of haddock. Moore (1938) compares the occurrence of four species of the genus *Bathyporeia* on a sandy beach in Scotland, showing their distinct zonation.

The family Caprellidae has already been mentioned as inhabiting the Caesarea rock-pools, but in the sublittoral zone it is represented by other species, *Pseudoprotella phasma f. bispinis* and *Phtisica marina* which are of an Atlantic-Mediterranean distribution and are on record from the Adriatic Sea (Fiorencis, 1942; Ruffo, 1946). *P. phasma f. typica* has also been found at the Egyptian coast near Alexandria.

THE PENETRATION OF AMPHIPODA THROUGH THE SUEZ CANAL

The collection of benthonic invertebrates from the Indian Ocean and Red Sea through the Suez Canal into the Mediterranean Sea has been proved for various groups. The order Crustacea, particularly the Decapoda, were first mentioned as forming an important migratory group (Monod, 1930). Indo-Pacific species of Decapoda are well represented in the eastern Mediterranean. Recently, more species have been added to the list as a result of the study of Israel Decapoda (Holthuis and Gottlieb, 1958).

The collection of Amphipoda from the eastern Mediterranean is not as comprehensive as that of Decapoda, but it is sufficient to indicate migration through the Suez Canal. The penetration of Amphipoda into the Suez Canal was first noticed by Schellenberg (1928) who studied the material collected by the Cambridge Expedition of 1924. Of the amphipods present along the Mediterranean coast of Israel, eight species were also found in the Suez Canal, of which only one, *Elasmopus pectenicrus*, has undoubtedly penetrated into the eastern Mediterranean from the Indo-Pacific region.

Perioculodes longimanus has not been found in the Suez Canal although it is present on both sides of this water-way, in the Atlantic-Mediterranean region and in the Indian Ocean including the Red Sea.

Hyale schmidti, which has been found in the Suez Canal, is known from the Atlantic-Mediterranean region and the Red Sea but not from the Indian Ocean. This distribution may suggest that *H. schmidti* has migrated from the eastern Mediterranean into the Red Sea. However, because of insufficient information concerning the distribution

A list of Amphipoda identified from the Mediterranean coast of Israel and their geographical distribution

		Atlantic Ocean	Western Mediterranean	Suez Canal	Red Sea	Indian Ocean	Pacific Ocean	Arctic Ocean
LYSIANASSIDAE	*Lysianassa ceratina* (A. Walker)	+	+	+	+	+	−	−
	Lysianassa cf. plumosa Boeck	+	+	−	−	−	−	−
	Lysianassa longicornis Lucas	−	+	−	−	−	−	−
	Socarnopsis crenulata Chevreux	+	+	−	−	−	−	−
AMPELISCIDAE	*Ampelisca brevicornis* (A. Costa)	+	+	+	+	+	+	−
	Ampelisca tenuicornis Lilljeborg	+	+	+	+	+	−	−
	Ampelisca sarsi Chevreux	+	+	−	−	−	−	−
	Haploops della-vallei Chevreux	−	+	−	−	−	−	−
HAUSTORIDAE	*Bathyporeia cf. guilliamsoniana* (Bate)	+	+	−	−	−	−	−
	Urothoe grimaldii Chevreux	+	+	−	−	−	−	−
PHOXOCEPHALIDAE	*Harpinia cf. pectinata* G. O. Sars	+	+	−	−	−	−	+
LEUCOTHOIDAE	*Leucothoe lilljeborgi* Boeck	+	+	−	−	−	−	−
OEDICEROSIDAE	*Westwoodilla cf. rectirostris* (Della Valle)	+	+	−	−	−	−	−
	Monoculodes subnudus Norman	+	+	−	−	−	−	−
	Perioculodes longimanus (Bate and Westwood)	+	+	+	+	+	−	−
ATYLIDAE	*Nototropis swammerdami* Milne-Edwards	+	+	−	−	−	−	+
GAMMARIDAE	*Gammarus locusta* Linné	+	+	−	−	−	+	+
	Echinogammarus olivii (Milne-Edwards)	+	+	−	−	−	−	−
	Eriopisa elongata Bruzelius	+	+	−	−	−	−	−
	Cheirocratus sundevalli (H. Rathke)	+	+	−	−	−	−	+
	Cheirocratus cf. assimilis Lilljeborg	+	+	−	−	−	−	−
	Megaluropus agilis Hoeck	+	+	−	−	−	−	−
	Maera grossimana (Montagu)	+	+	−	−	−	−	−
	Maera inaequipes (A. Costa)	+	+	+	+	+	+	−
	Elasmopus pocillimanus (Bate)	+	+	−	+	−	+	−
	Elasmopus pectenicrus (Bate)	+	−	+	+	+	+	−
DEXAMINIDAE	*Dexamine spinosa* (Montagu)	+	+	−	−	−	−	+
	Dexamine spiniventris A. Costa	+	+	−	−	−	−	−
TALITRIDAE	*Talitrus saltator* Chevreux	+	+	−	−	−	−	−
	Hyale schmidti (Heller)	+	+	+	+	−	−	−
	Hyale prevosti (M. -Edw.) *f. minor* (Chevreux)	+	+	−	−	−	−	−
AORIDAE	*Microdeutopus gryllotalpa* A. Costa	+	+	−	−	−	−	−
	Lembos cf. websteri Bate	+	+	−	−	−	−	−
	Lembos cf. longipes (Lilljeborg)	+	+	−	−	−	−	−
PHOTIDAE	*Leptocheirus cf. pectinatus* Norman	+	+	−	−	−	−	−
	Leptocheirus bispinosus Norman	+	+	−	−	−	−	−
AMPHITHOIDAE	*Amphithoe ramondi* Audouin	+	+	+	+	+	+	−
	Cymadusa filosa Savignyi	+	+	+	+	−	−	−
CAPRELLIDAE	*Caprella hirsuta* Mayer var. *longimana* Chevreux	−	+	−	−	−	−	−
	Caprella acanthifera Leach *f. grandimana* Mayer	−	+	−	−	−	−	−
	Pseudoprotella phasma (Montagu) *f. bispinis* Mayer	+	+	−	−	−	−	−
	Phtisica marina Slabber	+	+	−	−	−	−	−

of the fauna of the Indian Ocean, this cannot be accepted as evidence that *H. schmidti* has migrated through the Suez Canal.

The wide distribution of the following species: *Lysianassa ceratina*, *Ampelisca tenuicornis*, *A. brevicornis*, *Maera inaequipes*, *Cymadusa filosa* and *Amphithoe ramondi*, and their presence in the Suez Canal cannot be used to draw any definite conclusions as to their migration from one zoogeographic region to another.

ACKNOWLEDGEMENTS

The advice and criticism offered by Prof. K. Reich of the Hebrew University of Jerusalem in the course of this study are gratefully acknowledged.

The author wishes to express his thanks to Prof. H. Steinitz of the Hebrew University of Jerusalem for his valuable suggestions while this manuscript was being prepared for publication.

Dr. A. Yashouv (Wirszubski), the Fish Culture Research Station, Dor, generously placed at the author's disposal the collection of amphipods made during his benthos investigations (1946-1950).

The author also wishes to thank Prof. S. Ruffo, Museo Civico di Storia Naturale, Verona, Italy, for checking the identification of most of the species mentioned in this study.

REFERENCES

1. Barnard, K. H., 1937, *Amphipoda in the scientific reports of the J. Murray Expedition*, British Museum (Nat. Hist.), London, **4**, No. 6, 131–201.
2. Blegvad, H., 1930, Quantitative investigations of bottom invertebrates in the Kattegat with special reference to the plaice food, *Rep. Danish biol. Sta.*, Copenhagen, **36**, 55.
3. Chevreux, E. and Fage, L., 1925, Amphipodes, in *Faune de France*, Editor P. Lechevalier, 488.
4. Fiorencis, A., 1940, *I Caprellidi del mare Adriatico presso Rovigno*, Thalassia, Venezia, **4**, No. 6, 34.
5. Holthuis, L. B. and Gottlieb, E., 1958, An annotated list of the Decapod Crustacea of the Mediterranean coast of Israel, with an appendix listing the Decapoda of the eastern Mediterranean, *Bull. Res Counc. of Israel*, Jerusalem, **7B**, 120.
6. Jones, N. S., 1947, The ecology of the Amphipoda of the south of the Isle of Man, *J. Mar. biol. Ass. U. K.*, Plymouth, **27**, 400–439.
7. Monod, T., 1930, Über einige indo-pazifische Decapoden der Meeresfauna Syriens, *Zool Anz.*, **92**, 135–141.
8. Moore, H. B., 1958, *Marine ecology*, John Wiley, New York, 493.
9. Ruffo, S., 1936, Studi sui crostacei Anfipodi I. Contributo alla conoscenza degli anfipodi del Adriatico, *Boll. Ist. Ent. Univ. Bologna*, **9**, 23–32.
10. Ruffo, S., 1938, Gli Anfipodi marini VIII-IX, *Ann. Mus. Stor. nat. Genova*, **60**, 127–180.

11. Ruffo, S., 1941, Studi sui crostacei Anfipodi X, *Boll. Ist. Ent. Univ. Bologna*, **11**, 112–126.

12. Ruffo, S., 1946, Studi sui crostacei Anfipodi XI, Gli anfipodi bentonici di Rovigno d'Istria, *Boll. Soc. entom. ital. Genova*, **76**, 49–57.

13. Ruffo, S., 1950, Studi sui crostacei Anfipodi XXII, Anfipodi del Venezuela, *Mem. Mus. Civ. Stor. nat. Verona*, **2**, 49–65.

14. Ruffo, S., 1959, Contributo alla conoscenza degli Anfipodi del Mar Rosso, *Sea Fish. Res. Sta. Haifa, Bull.* No. 20, 11–36.

15. Schellenberg, A., 1928, Report on the Amphipoda collected during the Cambridge Expedition to the Suez Canal (1924), *Trans. zool. Soc. London*, **22**, 633–692.

16. Schellenberg, A., 1936, Amphipoda benthonica-Fishery Grounds near Alexandria, *Notes Fish. Res. Dir. Cairo*, No. 18, 27.

17. Soika, G., 1949, Gli Anfipodi Gammarini della laguna di Venezia, *Arch. Oceanogr. Limnol.* Roma, Anno 6, 165–212.

18. Watkin, E. E., 1939, A revision of the Amphipod genus *Bathyporeia* Lindström, *J. Mar. biol. Ass. U.K.*, Plymouth, **23**, 211–236.

19. Wirszubski, A., 1953, On the biology and biotope of the red mullet *Mullus barbatus* L., *Sea Fish. Res. Sta. Haifa, Bull.*, No. 7, 20.

LETTER TO THE EDITOR

REPRESENTATIVES OF THE ORDER AMBLYPYGI (ARACHNIDA) FOUND IN ISRAEL

R. ROSIN* AND A. SHULOV
Department of Zoology, The Hebrew University of Jerusalem

Four live specimens of the order: Amblypygi (class: Arachnida) were collected on separate occasions, between the 27th of March and 21st of April, during night hours; three of them inside a house (semi-cellar) in Jerusalem, the fourth on the exterior wall of the same house. Two of them, presumably adults, are 5.5 mm in body length, whereas the other two are not adults, only 2 mm long. The fact that different stages of development have been found suggests that these Arachnids breed in our country. They belong to the family Charontidae, as is evident by the presence of pulvilli at the end of the last three pairs of walking legs, and probably to the genus *Charinus*. Final identification is pending.

To the best of our knowledge, the presence of specimens of the order Amblypygi in Israel or any of the neighbouring countries has never been recorded. In fact J. Millot (1949) states that in Asia it was not to be found north of the Tropic of Cancer.

In his summary, J. L. Cloudsley-Thompson (1958) states that the Amblypygi are to be found only in the more humid regions of the tropics and subtropics. The order comprises two families only: Charontidae and Tarantulidae. The family Charontidae is composed of mostly small cavernicolous forms having a distribution in Southern and Eastern Asia, the Pacific Islands and Africa (J. Millot, 1949). A number of species of this family live in dark corners of houses in tropical regions. According to A. Kastner (1932), the nearest region to our country where the Charontidae are known to exist are the Seychelles and India; whereas of the Tarantulidae, which are still unknown in Israel, the subfamily *Phyrnicina* is to be found as near as Southern Arabia. Kastner suggests that Malaya might be regarded as the centre of dispersion of the Amblypygi in the Asian continent.

The Amblypygi are nocturnal and predatory in habits. Their anterior, relatively long, pair of legs function as antennae and are not used for walking. They are oviparous. The eggs are laid in a capsule which is attached to the ventral surface of the female's abdomen. When the young hatch they climb onto the mother's back and

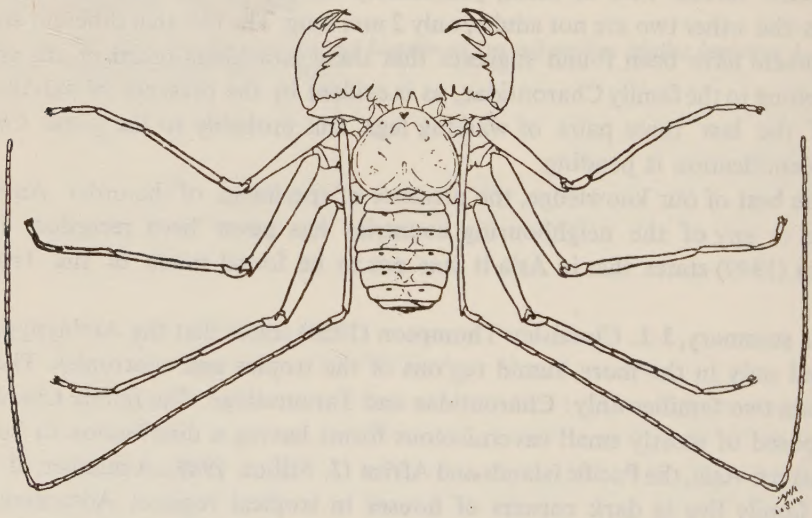
* Grant No E-2960 U.S.A., N.J.H.

Received May 20, 1960

remain there until after their first moult. The mating behaviour of this order is y unknown (Cloudsley-Thompson, 1958).

One of the specimens we found exhibits a certain asymmetry in the number segments of the fourth pair of walking legs. On the left leg the articulation betwe the first and second tibial segments is missing, and thus the tibia comprises only thr segments instead of the four found on the opposite tibia. The number of tibial segmen in the family Charontidae is usually four, rarely three (Kraepelin, 1899). The sam specimen shows also n asymmetry in the shape of the 8th opisthosomal tergite (s Figure).

Addendum: Since preparation of this note two more specimens have be collected, one (5 mm long, has been found at the same place mentioned abov another of the same size has been found by Mr. I. Chävatzelet under simil conditions.



Charinus sp. ? adult, dorsal view. Magnification: approx. X 20.

REFERENCES

1. MILLOT, J., Ordre des Amblypyges in P. P., *Grassés Traité de Zoologie*, Masson et Co., T. V 1949, 563.
2. CLOUDSLEY-THOMPSON, J. L., *Spiders, Scorpions, Centipedes and Mites, The Ecology and Natu History of Woodlice, "Myriapods" and Arachnides*, 1958, Pergamon Press, 124.
3. KASTNER, A., Ordnung der Arachnida: Pedipalpi Latreille in *Kükenthal Krumbach's Handbu der Zoologie*, **3**, 2, 1932, 1.
4. KRAEPELIN, K., *Scorpiones und Pedipalpi, Das Tierreich*, **8**, 1899, 235.

יוצא לאור ע"י
מוסד ויצמן לפרסומים במדעי הטבע ובטכנולוגיה בישראל
המועצה המדעית לישראל – משרד החנוך והתרבות – האוניברסיטה העברית בירושלים
הטכניון – מכון טכנולוגי לישראל – מכון ויצמן למדע – מוסד ביאליק

Published by
THE WEIZMANN SCIENCE PRESS OF ISRAEL
Research Council of Israel, Ministry of Education and Culture
The Hebrew University of Jerusalem, Technion—Israel Institute of Technology
The Weizmann Institute of Science, Bialik Institute

Printed in Israel
Raphael Haim Hacohen Press, Ltd., Jerusalem